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Developing a Brain-Based, Non-Invasive Treatment for Pain

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Developing a Brain-Based, Non-Invasive Treatment for Pain

by

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This work is dedicated to those that first taught me the value of knowledge

Irene and Leighton Dowdle

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Key to Abbreviations

Abbreviation	*Meaning*
ACC	anterior cingulate cortex
AFNI	Analyses of Functional Images
aMT	active motor threshold
ATS	Advanced Thermal Stimulator
BDI-II	Beck Depression Inventory II
BIS	Barratt Impulsiveness Scale
BPI	Brief Pain Inventory
BOLD	blood oxygen level dependent
CLBP	chronic lower back pain
COMM	Current Opioid Misuse Measure
cTBS	continuous theta burst
DLFPC	dorsolateral prefrontal cortex
EMG	electromyography
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
FWE	family wise error
FWHM	full width half max
gPPI	generalized psychophysical interaction
Hz	Hertz
ICA	independent component analysis
ICC	intraclass correlation
ICD	International Classification of Diseases
iTBS	intermittent theta burst
LTD	long term depression
LTP	long term potentiation

MI	primary motor cortex
MEP	motor evoked potential
MINI	Mini International Neuropsychiatric Inventory
MPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NAcc	nucleus accumbens
PCA	patient-controlled analgesia
PCA	principle component analysis
POMS	Profile of Mood States
PSQI	Pittsburgh Sleep Quality Index
QST	quantitative sensory testing
rMT	resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
SD	standard deviation
SEM	standard error of the mean
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SMA	supplemental motor area
SPECT	single photon emission computed tomography
SPM	Statistical Parametric Mapping
STAI	State Trait Anxiety Inventory
TBS	theta burst stimulation
TMS	transcranial magnetic stimulation

Abstract

LOGAN THORNE DOWDLE. Developing a Brain-Based, No-Invasive Treatment for Pain (Under the direction of COLLEEN A. HANLON).

Chronic pain cost society more than $500 billion each year and contributes to the ongoing opioid overdose crisis. Substantial risks and low efficacy are associated with opiate usage for chronic pain. This dissertation seeks to fill the urgent need for a new pain treatment using a neural-circuit based approach in healthy controls and chronic pain patients.

First, we performed a single-blind study examining the causal effects of transcranial magnetic stimulation (TMS), compared to a well-matched control condition. Using interleaved TMS/fMRI we explored brain activation in response to dorsolateral prefrontal cortex (DLPFC) stimulation in 20 healthy controls. This study tested the hypothesis that the TMS evoked responses would be in frontostriatal locations. Consistent with this hypothesis active TMS, compared to the control, led to significantly greater activity in the caudate, thalamus and anterior cingulate cortex (ACC).

Building on these findings, we developed a single-blind, sham-controlled study examining two TMS strategies for analgesia in 45 healthy controls. We completed an fMRI thermal pain paradigm before and after modulatory repetitive TMS at either the DLPFC or the medial prefrontal cortex (MPFC). Despite a role in pain processing, the MPFC has not yet been explored as a target for analgesia. Only MPFC stimulation significantly improved behavioral pain measures. These effects were associated with increased motor and parietal cortex activity during the pain task.

We then supplement these findings by testing the hypothesis that chronic pain patients who use opioids (n=14) would have elevated brain responses to thermal pain relative to healthy controls (n=14). Despite indistinguishable self-report measures, we found increased brain activity in the ACC and sensory areas in patients which were positively correlated with opioid dose.

We conclude by evaluating the feasibility of these approaches in chronic pain patients, reporting preliminary findings from a pilot study examining the two treatment strategies tested previously in controls. Collectively, our findings support a circuits-first approach to pain treatment. Though MPFC stimulation was effective in reducing pain in healthy controls, further work is required to confirm these results in a chronic pain population, as chronic pain and opioid usage alter how the brain processes the pain experience.

Chapter 1 The Case for a Neural Circuit Based, Non-Invasive Treatment for Pain

Pain in the Brain

When your hand touches a hot stove there is a nearly instantaneous personal experience of pain, with its attendant localization, sensations, and feelings. In order to discuss pain and pain processing, it is first necessary to disentangle it from nociception, the signaling cascade that occurs in those first few moments after touching the stove. Nociception is an aspect of sensory systems that allows an animal to detect and rapidly react to a noxious stimulus (Treede 2006) – such as the hot stove. After hand contact activates heat sensitive nociceptors in the finger tips, action potentials will travel along what are known as the first order, C and A-delta fibers. These synapse on the dorsal horn of the spine. The majority of second-order projections from the dorsal horn cross the midline and ascend, forming the spinothalamic tract, to target neurons located in the lateral and medial thalamus. Finally, third order projections from the thalamus target a variety of cortical regions. Up until this point, there has been no perception of pain, though reflexes may have already removed the hand from the stimulus. Only with cortical processing does the nociceptive cascade get interpreted, undergoing a transition from informative signal into a multifaceted conscious experience. At this moment we can choose a reaction, pulling our hand back from the heat to prevent further damage. Though the nociceptive signal arises from specialized receptors spread throughout the body, the pain experience occurs within the brain.

The idea of pain within the brain has historical precedence, though anatomical accuracy has been obtained only somewhat recently. Historically, the conscious experience of pain was a subject of continuous philosophical enquiry. in the 17th century, Descartes has the distinction of correctly identifying that the brain was a target of pain, however he incorrectly thought pain projections terminated at the pineal gland (Descartes 1649) -- the seat of the soul ("le siège de l'âme"). This idea, that pain was a something felt, rather than a sensory experience was compelling, and correctly captured important affective aspects that are associated with pain. In the early 20th century, however, thought shifted to regard pain as sensory experience, something that could be measured with the contemporary physiological techniques. During this period, the thalamus was regarded as the integrator of pain and responsible for the pain sensation (Head and Holmes 1911). Only in the in the last century have the separate threads of thought joined with emerging cognitive theories into a more complete picture of the pain experience and localized the pain experience to the cortex (Treede, Kenshalo et al. 1999). Though the thalamus is necessary to convey the nociceptive information, only in the cortex does the signal undergo sufficient processing to produce the pain experience (Garcia-Larrea and Peyron 2013, Bastuji, Frot et al. 2016).

This description of pain as existing within the brain in specific but distributed cortical areas is a change from the pioneering work of Penfield and Boldrey. Using direct electrical stimulation of the exposed surface of the human cortex, they sought to map out sensory and motor representations by eliciting small movements or sensations (Penfield and Boldrey 1937). Despite decades of experimental work they did not uncover a pain area. In their report they instead stated "the fact that only eleven times out of well over 800 responses did the patient use the word pain to describe a cortical sensation, probably indicates that pain has little, if any,

true cortical representation." (Penfield and Boldrey 1937). Furthermore, any painful responses identified were attributed to referred pain, due to direct stimulation of the organ. These findings highlight a rather unique feature of pain sensation. In contrast to the representations of vision, somatosensation and motor output, there is no large, single topographical region responsible for pain processing. More recent work with electrical stimulation has found that pain sensations can be elicited by cortical stimulation, but only in very specific locations, such as the secondary somatosensory cortex and the posterior insula (Mazzola, Isnard et al. 2012).

There are two connected components to understanding the pain experience. The first is to categorize its various aspects and understand the role of the larger context in which the pain is occurring, such as mood, cognition or injury. The second component seeks to identify the underlying anatomical and functional substrates of these experiences. This manner of thinking emerged from the concept that distributed brain areas with specialized functions are integrated on order to provide a conscious experience, in this case -- pain (Melzack and Casey 1968). This was developed more broadly into the idea of the "neuromatrix" from research on phantom limbs (Melzack 1989, Melzack 1990). Notably the idea of the neuromatrix was non-specific to pain, and instead referred to the integration of the sensory inputs with internal information, such as affect, and memories to generate the sense of self. More recently the term evolved to become the "Pain Matrix", referring the distributed regions that process pain (Iannetti and Mouraux 2010). Before exploring how functional neuroimaging has contributed to the idea of the pain matrix and each region's relative contribution to pain, we will take a closer examination of the components of the experience, which can be broadly divided into three relatively unique parts. These are the sensory-discriminative, affective-motivation and the cognitive-evaluative components discussed by Melzack in 1968. Over time the classifications have become more

detailed and linked with additional behaviors, but these three components are sufficient to describe acute pain.

If we once again place our hand on the stove, we can identify contributions from each system in the personal and unified experience. Sensory-discriminative systems determine the 'where and what'. This system serves to identify that the pain is in our hand, its relative intensity and classify the stimulus as heat. Affective-motivation systems are reflected in our thoughts that the pain is uncomfortable, and the pressing urge to remove our hand from the heat as fast as possible. Cognitive components capture a wide range of processes that can modify the intensity of the pain. For example, if we had taken a pill that we believed to be a powerful analgesic (placebo) or had the expectation that the stove was much hotter, then we may experience less pain upon touching it. In contrast, the expectation that the stove was off would bias our pain experience in the opposite direction. Recent work using neuroimaging has allowed an investigation of the underlying anatomy (Figure 1.1) that supports these processes using careful manipulations with acute pain.

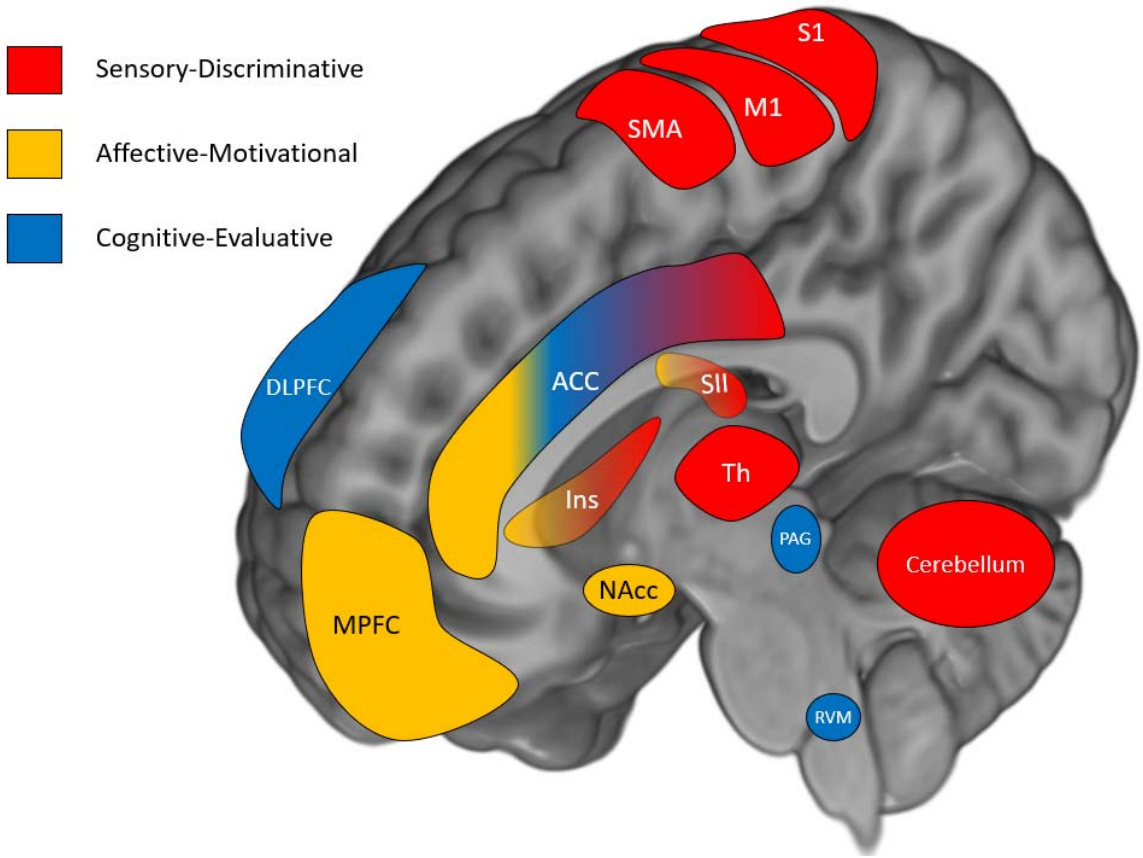

Figure 1.1 Brain areas associated with pain processing. Nociceptive projections ascend from the spine to the thalamus and from there project to multiple areas in the brain. Regions involved in the pain response can be broadly classified into sensory-discriminative (Red) that identify the location and type of pain; affective-motivational areas (Yellow) that deal with emotion processing and motivated responses as well as cognitive-evaluative (Blue) areas that regulate the pain experience. Some areas perform multiple functions, here indicated by blending colors. RVM – Rostral Ventromedial Medulla, APG - Periaqueductal Gray, NAcc - nucleus accumbens, Th – thalamus, Ins - insula, SI - primary sensory cortex, SII – secondary sensory cortex, SMA – supplementary motor area, MI – motor cortex, DLPFC – dorsolateral prefrontal cortex, MPFC – medial prefrontal cortex, ACC – anterior cingulate cortex.

When using neuroimaging, there are a large number of areas associated with pain (Martucci and Mackey 2018) (Figure 1.1), with the most commonly reported areas of increased activation in thalamus, insulae, primary (SI), secondary somatosensory (SII) cortices, and the anterior cingulate cortex (ACC) (Apkarian, Bushnell et al. 2005, Tracey and Mantyh 2007). This activation is reflected by increases in the blood oxygen dependent signal (BOLD) signal, and occurs in response to thermal (Disbrow, Buonocore et al. 1998, Becerra, Breiter et al. 1999, Derbyshire, Jones et al. 2002, Brown, Chatterjee et al. 2011), mechanical (Disbrow, Buonocore et al. 1998, Cauda, Costa et al. 2014), chemical (Maihofner and Handwerker 2005) and other types of painful experimental interventions (Pogatzki-Zahn, Wagner et al. 2010).

Sensory-Discriminative

That these diverse methods of eliciting pain have common neural representations is due in part to the common pathway of nociceptive signals through the thalamus. The division of targets of first order fibers to lateral (i.e. ventroposterior lateral) and medial (i.e. mediodorsal) thalamus has historically given rise to a categorization of lateral and medial pain systems (Albe-Fessar, Berkley et al. 1985). With improving neuroimaging techniques this dichotomy has been superseded, however the broad rules of processing remain accurate. The targets of the lateral projections from that thalamus, primarily the primary (SI) and secondary (SII) somatosensory cortices are responsible for the sensory-discriminative aspects of pain processing and relate the location and identification (ex. hot, cold, prickling) of the stimulus (Chen, Ha et al. 2002, Apkarian, Bushnell et al. 2005, Lee and Tracey 2010). SII, as well as insula, and posterior portions of the ACC show relationships with the intensity (i.e. temperature) of the stimulus (Coghill, Sang et al. 1999, Büchel, Bornhövd et al. 2002). Motor regions, including the primary motor cortex

(M1) and the cerebellum are also recipients of this information, in order to execute an action to remove the body from harm.

Affective-Motivational

The anterior cingulate, insula and SII also process information related to affect and motivation, for example relating the unpleasantness of the painful stimulus (Rainville, Duncan et al. 1997, Price 2000, Apkarian, Bushnell et al. 2005). The ACC reflected increases and decreases In individuals hypnotized and instructed to regulate unpleasantness up and down respectively (Rainville, Duncan et al. 1997). The medial prefrontal cortex regulates how pain is evaluated (Leknes, Berna et al. 2013), particularly when it is altered by context (Roy, Shohamy et al. 2012). Relative increases of activity within the MPFC is often associated with decreased pain (Reddan and Wager 2018), and this effect can be manipulated. For example if a participant elects to experience pain in place of their partner, this increased activity occurs with participants reporting less pain (Lopez-Sola, Koban et al. 2018).

Cognitive-Evaluative

For cognitive control and regulation of pain there are additional regions, such as the dorsolateral prefrontal cortex (DLPFC), nucleus accumbens (NAcc) periaqueductal gray (PAG) and rostroventral medulla (RVM) involved, in addition to portions of the anterior cingulate cortex. The DLPFC, NAcc, PAG and RVM are heavily involved in the reduced painfulness associated with placebo (Wager, Rilling et al. 2004, Eippert, Bingel et al. 2009, Zubieta and Stohler 2009). The brainstem regions project to the spinal cord, effecting pain relief (Eippert, Finsterbusch et al. 2009). This can be blocked by naloxone (Eippert, Bingel et al. 2009)

suggesting a role for endogenous opioids. The ACC also appears to play a role in cognitive regulation of pain, with increased activation during distraction (Bantick, Wise et al. 2002), corresponding with lower pain.

In giving rise to this distributed model of pain processing in 1968, Melzack stated "In a model such as this, 'function' does not reside in any one area. Rather each specialize portion of the brain contributes to experience and response as a whole". This conjecture has been recently confirmed with further advances in neuroimaging that capture the signature of pain (Wager, Atlas et al. 2013). Critically this model is most accurate when applied to whole brain data, such that every area of the brain contributes to the model prediction (Wager, Atlas et al. 2013, Woo, Roy et al. 2015, Zunhammer, Bingel et al. 2018). It is important at this point to note that, though the pain matrix regions are sensitive to aspects of the pain experience, they are not specific. Many of these same regions show common patterns of activation to viewing others in pain (Ochsner, Zaki et al. 2008), or respond to any salient stimulus, regardless of its sensory modality (Mouraux, Diukova et al. 2011). As researchers seek to identify the presence of pain within the brain, these limitations become ever more important.

Chronic Pain

While the above description of pain processing is sufficient for a painful event such as touching a hot stove or a stubbed toe, it does not fully explain the process of chronic pain - defined as pain persisting for more than 3 months (Treede, Rief et al. 2015). Chronic pain affects over 25 million adults on a daily basis, with more than 125 million endorsing current acute or chronic pain in the past 3 months (Nahin 2015, Borsook, Youssef et al. 2018). This persistent

pain leads to high societal costs, recently estimated at over $500 billion (Gaskin and Richard 2012).

The etiology of chronic pain is highly varied, with the recent International Classifications of Diseases report (ICD-11) identifying 7 subtypes (Treede, Rief et al. 2015). These classifications cover a wide range from neuropathic pain caused by a lesion in the sensory nervous system (ex. phantom limb pain, pain from stroke), to postsurgical or posttraumatic pain and on to chronic primary pain, which includes back pain and fibromyalgia (Treede, Rief et al. 2015). Each of these classifications involves different pain signaling processes. For example, neuropathic pain can be caused by a nerve sending a tonic pain signal, which is then experienced as continuous pain in the absence of an external noxious stimulus (Costigan, Scholz et al. 2009). Chronic pain is not limited to maladaptive signals from the periphery, but can also be the result of plastic changes in the brain and spinal cord that amplify incoming nociceptive signals, a phenomenon known as central sensitization (Latremoliere and Woolf 2009). This can lead to previously non painful stimuli becoming painful (allodynia) or increased sensitivity to painful stimuli (hyperalgesia).

One common and frequently studied cause of chronic pain is chronic low back pain (CLBP), which can fall into multiple ICD categories, including chronic primary pain, musculoskeletal or neuropathic pain. Regardless of its source, it is a large health burden and is associated with the largest number of years lived with disability (Murray, Atkinson et al. 2013). As it is well studied, CLBP servers as an example of the information that neuroimaging can provide about pain. In line with the idea that chronic pain is associated with brain plasticity, there is evidence that CLBP can is associated with reduced brain volume, particularly in prefrontal regions (Apkarian, Sosa et al. 2004, Schmidt-Wilcke, Leinisch et al. 2006, Seminowicz, Wideman et al. 2011, Ivo, Nicklas et al. 2013). With a longer duration of chronic pain there appears to be a progressive loss of gray

matter in the left dorsolateral prefrontal cortex (Barad, Ueno et al. 2014), however there is evidence that effective treatments can reverse this decline. (Seminowicz, Wideman et al. 2011, Rodriguez-Raecke, Niemeier et al. 2013). These structural changes are present alongside differences in functional brain activity as well. In examining functional connectivity in CLBP, there are alterations, relative to healthy controls, in the functional connectivity of the brain in many of the areas of the pain matrix (Kregel, Meeus et al. 2015) including the MPFC (Baliki, Geha et al. 2008, Buckalew, Haut et al. 2010, Baliki, Baria et al. 2011, Baliki, Petre et al. 2012, Hashmi, Baliki et al. 2013) anterior cingulate cortex (Baliki, Geha et al. 2008, Hashmi, Baliki et al. 2013, Kornelsen, Sboto-Frankenstein et al. 2013) and insula (Tagliazucchi, Balenzuela et al. 2010, Kornelsen, Sboto-Frankenstein et al. 2013). These changes in connectivity may not be epiphenomenal, but instead may be predictive of the development of chronic pain (Baliki, Petre et al. 2012, Apkarian, Baliki et al. 2013).

Despite these promising findings, there is limited evidence on how the brain, and in particularly the functional responses of the brain change to pain in the presence of one of the most common treatments – prescription opioids. A recent meta-analysis demonstrated brain response to acute pain in individuals with chronic pain has similar spatial characteristics to control groups (Tanasescu, Cottam et al. 2016), however the majority of these studies excluded patients who use opioids, and meta-analyses such as this cannot examine the magnitude of the BOLD response, but only estimate the extent. Before discussing prescription opioids, the next section will examine the endogenous opioids produced by the body. Given the prevalence of opiate usage even among individuals with chronic pain, there are important unanswered questions regarding how these pharmaceutical agents alter unaided pain processing mediated by endogenous opioid peptides.

The Endogenous Opioid System

Our bodies produce opioid peptides internally in response to a wide range of stimuli. These peptides (beta-endorphin, dynorphin and enkephalin) are present throughout the central nervous system. They bind at mu, delta and kappa opioid receptors. In particular, the mu opioid receptor is associated with pain relief and reward. While both enkephalin and beta-endorphin can bind at the mu receptor, beta-endorphin has the highest affinity and preferentially binds there to mediate its pain-relieving effects.

The discovery of endogenous opioids was preceded by the use of morphine and other natural and synthetic opiates. The distribution and binding sites within the human brain were determine using Positron Emission Tomography (PET), which uses ligands for opioid receptors that are radioactively tagged. As these tagged ligands, known as radiotracers, bind at endogenous sites they release positrons, which can be detected by the PET camera. This can be performed at rest, or in conjunction with a task. A relevant example of this would be comparing a baseline rest state with pain, whilst using an opioid receptor sensitive ligand. This reveals that, during pain, endogenous opioids are released that then bind and displace the external radiotracer in critical areas of the pain network, including the ACC and insula (Sprenger, Valet et al. 2006).

As expected from the specific regions mentioned above, these receptors are not uniformly enriched, but instead have their highest concentrations within certain locations. The thalamus and insula contain the highest concentration of opioid receptors, when measured using a nonselective agonist (Baumgartner, Buchholz et al. 2006). For the mu opioid receptor, the thalamus and brainstem are most heavily enriched, whereas prefrontal regions have a more

uniform distribution of mu and delta receptors (Corder, Castro et al. 2018). In regards to analgesia, the mu opioid receptor receives substantial attention, as it is tightly linked with both the pain relieving and rewarding aspects of opioids. The kappa opioid receptor also has a role in pain, though activation tends to oppose the effects of the mu receptor (Pan 1998) and activation of the kappa system itself is associated with dysphoria (Land, Bruchas et al. 2008). For these reasons, antagonists for the kappa system are currently being explored as possible treatments for withdrawal following opioid abstinence (Zan, Wang et al. 2015).

Delta opioid receptors also mediate pain responses and are currently being explore as a potential alternative to the predominantly mu targeting prescription opiates (Spahn and Stein 2017). The delta receptors also have an extensive role in emotion processing, unique from that of the mu receptor. The effects are well characterized in animal models. For example, in mice, genetic deletion of the delta opioid receptor increases anxiety-like behaviors (Filliol, Ghozland et al. 2000), as does the use of a delta antagonist in rats (Perrine, Hoshaw et al. 2006).

Collectively these receptors and peptides regulate multiple aspects of human physiology and behavior. Though they are most well-known and identified with pain, they are also the underlying system that is misappropriated by drugs of abuse that mediate their actions through rewarding properties. At the intersection of both concerns are the pharmacologically developed opiates, particularly those that target the mu opioid receptor subsystem.

Current treatment for acute pain: prescription opioid medication

Opiates are an extract from the poppy, *papaver somniferum*. The poppy itself, and its seeds have been used since antiquity as a food source, medicine and for ritual purposes (Nencini 2009). In regards to medicinal usage, there is extensive evidence that the opium was a valuable

part of the pharmacopeia, perhaps as early as the 8th century B.C.E. (Julyan and Dircksen 2011). While early cultures used crude extracts from the poppy for medicinal purposes, current methods of drug discovery have led to entirely new and powerful synthetic and semisynthetic opiates.

Currently the pharmacologically derived opiates are widely prescribed for the treatment of pain, with 245 million prescriptions written in 2014 (Volkow and McLellan 2016). While opioids are highly effective for acute pain and are a critical tool, there are a number of concerns associated with their long-term use. Despite wide spread prescribing practices there is a lack of support for the use of opioids in chronic pain (Chaparro, Furlan et al. 2013, Chou, Turner et al. 2015).

In addition to low efficacy, high rates of tolerance and abuse liability pose serious risks for patients using these drugs long term. Tolerance is a condition in which a larger dose of the drug is required in order the get the same effect. This can be a particular concern with opioids given that there is a risk of respiratory depression or other dangerous off-target effects as dosage increases. Tolerance to opioids has been ascribed to a number of different causes (Kim, Stoicea et al. 2014), including receptor internalization (He, Fong et al. 2002), receptor down regulation (Stafford, Gomes et al. 2001), and pharmacodynamic environmental responses (Siegel 1976).

An additional possible contribution to opioid tolerance is hyperalgesia, in which pain sensitivity is increased. Paradoxically, individuals taking prescription opiates can develop opioid induced hyperalgesia (Angst and Clark 2006, Chu, Angst et al. 2008, Arout, Edens et al. 2015). This presents difficulties for clinicians trying to determine the optimal dose for pain relief. Hyperalgesia can develop to the original painful stimulus, such as chronic back pain becoming more severe following long-term opioid usage, or it can leave patients more sensitive to other

pain. While opioid induced hyperalgesia cannot fully explain the occurrence of tolerance, it is harmful in and of itself, and more probable as dosages of opiates increase. In some cases, tapering the dose of opioids to zero may be recommended as a treatment for opioid induced hyperalgesia (Lee, Silverman et al. 2011).

With increasing opioid dosage there is also an increased risk of opioid use disorder. For some time it was thought that ongoing pain was protective against the development of addictive behaviors (Colpaert, Meert et al. 1982, Lyness, Smith et al. 1989, Colpaert, Tarayre et al. 2001, Ozaki, Narita et al. 2004). More recently, evidence is growing to show that this may not be the case (Ewan and Martin 2013, Zhang, Tao et al. 2014, Hou, Cai et al. 2015). There is now epidemiological evidence of misuse and addiction even among individuals with chronic pain (Vowles, McEntee et al. 2015). As dose and duration of opiate usage increase, the risk of opiate use disorder in substantial, with high dosage, chronic use increase the risk by a factor of 140 (Edlund, Martin et al. 2014). Among a population of individuals with opiate use disorder, nearly 80% had a prescription, and among the remainder, 50% had a family member with an opiate prescription (Shei, Rice et al. 2015). Many people transition to heroin usage due to cost or availability and individuals who use heroin are now 10 times more likely to say their first usage was via prescription opiates (Compton, Jones et al. 2016). In general, the increased availability of opiates contributes to higher rates of misuse (Compton and Volkow 2006), and there is a risk that abuse deterrent technology may be driving more harmful heroin usage (Dart, Surratt et al. 2015). As addiction risk and higher dosages prevail there is an increased risk of opioid related overdoses, which are often fatal. In 2017 there were over 40 thousand fatal overdoses, an increase of 12% from the previous year (Scholl, Seth et al. 2018). Current recommendations from the Centers for Disease Control recommends that a non-opioid treatment be tried prior to

using opioids (Dowell, Haegerich et al. 2016) and work is ongoing to reduce overdose occurrence.

Advancing treatment options for acute pain

Developing a neural-circuit based intervention

There is an urgent need for a new, non-pharmacological treatment for pain. This issue has been recognized by the Centers for Disease Control, which recommends a non-opioid first response for treatment (Dowell, Haegerich et al. 2016). Clinicians have turned to a series of other options, which grow increasingly important considering the opioid overdose crisis. These include mindfulness meditation (Orme-Johnson, Schneider et al. 2006, Zeidan, Martucci et al. 2011, Zeidan, Grant et al. 2012, Zeidan, Emerson et al. 2015), cognitive behavioral therapy (Jensen, Kosek et al. 2012, Shpaner, Kelly et al. 2014) and other pharmacological agents, such as antidepressants (Mico, Ardid et al. 2006, Lee and Chen 2010). While these options are associated with less overdose risks or abuse potential, this document argues for a more direct and brain-based approach. The brain systems associated with pain (see Figure 1.1) may be amenable to modulation using non-invasive brain stimulation. Many methods of chronic pain require an extensive time commitment or high levels of motivation for each individual patient. For example, with cognitive behavioral therapy, activities outside of therapy sessions ('homework') is emphasized as a key component for effectiveness (Johnson and Kazantzis 2004, Kazantzis, Arntz et al. 2012), however, surveys of clinicians administering CBT find nonadherence rates as high as 50% (Helbig and Fehm 2004, Gaynor, Lawrence et al. 2006).

Targeting the brain regions directly, rather than via individual effort may prove to be a more effective strategy to attenuate pain.

While there are multiple pharmacological treatments for pain that vary in effectiveness, there is no FDA approved tool that targets pain where it is experienced – within the brain. The next section will introduce one method by which researchers and clinicians can target the cortex of the human brain, thereby measuring and manipulating human brain activity.

Introduction to Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive human brain stimulation tool. TMS is produced by running current through 100-200 circular windings of metal wire which are encased in a plastic or otherwise magnetically permissible housing. In order to produce the magnetic field, brief pulses of current are passed through the coil. Consistent with Maxwell's third law, these time varying electrical currents produce magnetic fields that also vary over time – which in turn can produce electrical currents perpendicular to the magnetic field (Maxwell 1861). If a conductive biological substrate is placed in this time varying magnetic field, then an electrical current will be produced. A clear example of this involves placing the TMS coil over the nerves or muscles of the arm. With each magnetic pulse, a muscle twitch will be observed (Barker, Jalinous et al. 1985). When the amplifier output is reduced, or if the coil is moved away from the arm, the twitches will reduce in magnitude. This is due the rapid fall off of the strength of the magnetic field, which decreases exponentially (Bohning, Pecheny et al. 1997, George, Bohning et al. 2007).

For *transcranial* magnetic stimulation the pulse is delivered through the scalp. The magnetic field passes directly through the skull and dura, with the only decay due to distance, rather than

resistance as is the case in electrical stimulation. Historically, there were multiple attempts to observe interactions between magnetic fields and the neural tissue of the brain (D'Arsonval 1896, Thompson 1910), however, measurable effects were only reported for peripheral nerve stimulation (Hallgren 1973, Polson, Barker et al. 1982). Inspired by this early work, Dr. Anthony Barker, then a doctoral student at the University of Sheffield, explored magnetic stimulation and obtained only 'slight effect[s]', and returned to his doctoral research on a separate topic (Freeston 1994). Returning to magnetic stimulation in 1984, Dr. Barker then published the seminal work on transcranial magnetic stimulation, applying single pulses of TMS to the left primary motor cortex using a circular coil (Barker, Jalinous et al. 1985). A movement was detected in the right hand, which was proportional to the strength of the magnetic field. This elegantly demonstrates the principles and potential of magnetic stimulation. While early TMS devices were circular, the most common design is currently a figure 8 shaped coil, though a wide variety of shapes have been considered in order to produce more focality or depth (Deng, Lisanby et al. 2013).

The figure 8 coil is able to directly stimulate a region of the cortex that is roughly 12.5 cm^2 (Hanlon 2016). In practice, the peak electric field is thought to be considerably more focal (Thielscher, Antunes et al. 2015), such that twitches can be induced in individual muscles, causing the movement of single fingers. This movement can be quantified by measuring the peak-to-peak electrical activity in the muscle with electromyography (EMG). This TMS motor evoked potential (MEP) provides researchers with a clear dependent measure to explore the effects of TMS.

Using single pulse TMS as a measurement tool

Single pulses of TMS can be used to evoke activity in a circuit. The motor circuitry is a common target, as the motor evoked potential (MEP) produced by TMS is readily measurable. These TMS evoked MEPs are a multi-synaptic process, from the cortex, to the spine, and then from the spine to the muscle corresponding to the stimulation site. The magnitude of these MEPs at a given machine output is highly reliable with a given individual (Intraclass correlation (ICC) values >= 0.60) (Kamen 2004, Malcolm, Triggs et al. 2006, Bastani and Jaberzadeh 2012). Between participants, however, there is large variability. For example, in a population of 189 individuals with depression, the machine output required to generate a thumb twitch ranged from 26 to 95% (George, Lisanby et al. 2010). In order to compare groups and have a common metric for analyses the field has developed the resting motor threshold (rMT). The rMT is defined as the minimum TMS amplifier output required to elicit a muscle activation 50% of the time. This metric provides a normalizing measure for variability across the population. Notably this variability isn't an easy measure of neuronal excitability or some other physiological process. The majority of the variability is simply due to magnetic field decay, as approximately 70% of the variability in the rMT is due to scalp to cortex distance (Herbsman, Forster et al. 2009).

Using repetitive TMS (rTMS) as a modulatory tool

When TMS is delivered at a specific frequency over a period of time, it has the capability to induce effects that mimic long term potentiation (LTP) or long term depression (LTD) in the targeted circuit. This method is known as repetitive TMS (rTMS). These effects are frequency dependent, in that higher frequencies (>5Hz) tend to lead to LTP-like effects (Berardelli,

Inghilleri et al. 1998, Maeda, Keenan et al. 2000), whereas lower frequencies (1Hz) tend to promote LTD-like effects (Chen, Classen et al. 1997, Maeda, Keenan et al. 2000, Gerschlager, Siebner et al. 2001) (reviewed in (Fitzgerald, Fountain et al. 2006, Thickbroom 2007)). In contrast the use of single pulse TMS to measure circuits of interest, rTMS is a full-fledged clinical treatment. rTMS has been FDA approved for the treatment of depression since 2008, following positive findings from clinical trials (O'Reardon, Solvason et al. 2007). Building on this foundation, rTMS is now being explored as a tool for addiction (Barr, Farzan et al. 2011, Bellamoli, Manganotti et al. 2014, Gorelick, Zangen et al. 2014, Dunlop, Hanlon et al. 2016, Diana, Raij et al. 2017), autism (Barahona-Correa, Velosa et al. 2018), and many other neuropsychiatric disorders (Blumberger, Barr et al. 2015).

rTMS Fundamentals.

The effects of rTMS are best understood through the study of the motor system, which elegantly demonstrates the effects of stimulation by increases or decreases in the size of the MEP. Early work in the field demonstrated that the size of MEPs could be increased after just 20 pulses at 10 or 20Hz (Pascual-Leone, Valls-Sole et al. 1994) though these effects lasted only 4 minutes. Using 5Hz stimulation for 8 minutes (1800 pulses), the increased MEPs persisted for up to 30 minutes (Peinemann, Reimer et al. 2004). Effects in the opposite direction were also found, with 15 minutes of 0.9Hz stimulation (810 pulses) delivered to the motor cortex leading to a reduction in the size of the MEP for 15 minutes (Chen, Classen et al. 1997). Since this early work there has been extensive research investigating the duration of the effects, the number of pulses required and other parameters. One recent advance has centered around using particular patterns of pulses, shifting away from the use of just a single frequency.

Theta Burst Stimulation

In recent years, considerable effort has been expended on discovering new stimulation paradigms that have greater efficacy or faster mechanisms of action. One example is theta-burst stimulation (TBS). In preclinical research theta-burst stimulation is a heavily used and well characterized method of electrical stimulation that reliably produces long term potentiation (LTP) or depression (LTD) of brain activity (Bear and Malenka 1994, Otani, Blond et al. 1998, Urban, Kossut et al. 2002, Malenka and Bear 2004). These methods were adapted for TMS and applied to humans for the first time by Dr. Jonathan Rothwell's laboratory at the University College-London (Huang and Rothwell 2004). Human theta burst stimulation is typically performed by delivering a series of 50Hz triplets – the eponymous 'burst'- to the cortex at 5Hz. When these bursts are delivered one after another at 5Hz, this method is known as continuous TBS (cTBS). When delivered with breaks, this method is known as intermittent TBS (iTBS). iTBS typically is performed with a 2 second period of stimulation followed by an 8 second period of no stimulation. When delivered for 600 total pulses, cTBS and iTBS have been shown to introduce LTD-like or LTP-like effects on the motor cortex (Di Lazzaro, Pilato et al. 2005, Huang, Edwards et al. 2005).

At this dose (600 pulses) 40 seconds of cTBS has been shown to produce effects of a similar magnitude to 4 minutes of 1 Hz, with reductions in MEP size of 45% compared to 1Hz effects at 34.03% (Maeda, Keenan et al. 2000, Huang, Edwards et al. 2005). The duration of effects appears to be greater, as 40 seconds of cTBS led to at least 60 minutes of MEP suppression compared to 15 minutes following 15 minutes of 0.9Hz stimulation (Chen, Classen et al. 1997, Huang, Edwards et al. 2005).

Due to the breaks introduced during iTBS stimulation, 600 pulses of iTBS lasts 3 minutes and 9 seconds. This remains a much shorter stimulation period relative to typical 10 Hz protocols, which often last for 20 to 30 minutes. Despite this shorter duration, the effects on the magnitude of the MEP are also comparable, with 75% increases in MEP size after 600 pulses, compared to approximately 38% after 10Hz stimulation (Maeda, Keenan et al. 2000, Huang, Edwards et al. 2005). For iTBS the increased MEPS were present for at least 15 minutes (Huang, Edwards et al. 2005), though effects may persist out to 1 hour (Gamboa, Antal et al. 2011) which is of a similar duration to 10 Hz protocols (Klomjai, Katz et al. 2015).

cTBS effects beyond the motor system

While the motor system has been a consistent source of information regarding TMS effectiveness, there are additional sources that support using the biologically informed, faster methods. Prior work from our laboratory has shown that a session of cTBS is able to reduce the BOLD response to single pulses of TMS in a cohort of alcohol and cocaine users (Hanlon, Dowdle et al. 2017). Across both cases, the response to single pulses of TMS was attenuated in the region beneath the coil, as well as in the insula. Diving deeper into these effects, our lab has shown that these effects extend to task processing. Specifically, a single session of active cTBS, relative to sham, altered correlations during drug cue processing between the target site and connected areas (Kearney-Ramos, Dowdle et al. 2018). Though this addiction related sample differs from healthy controls or individuals with chronic pain, this suggests that cTBS to the frontal pole, or medial prefrontal cortex, may be an effective tool to reduce activity in areas typically associated with pain.

iTBS effects beyond the motor system

One of the key benefits of theta burst stimulation is the shorten duration of each TMS session which can reduce the cost and time burden for patients. For treatment resistant depression iTBS appears to be equivalent to 10Hz stimulation, leading to similar response rates of approximately 50% whether targeting the dorsomedial prefrontal cortex (Bakker, Shahab et al. 2015) or dorsolateral prefrontal cortex (Blumberger, Vila-Rodriguez et al. 2018). That these treatments only take 3 minutes and 9 seconds in comparison to the traditional 30 minute, FDA approved protocol opens entirely new possibilities. For example, multiple treatments can more easily be delivered over a single day, which may produce larger effects (Nyffeler, Wurtz et al. 2006, Cazzoli, Muri et al. 2012). In a small sample of highly treatment resistant individuals, a recent study found that 10 daily sessions of iTBS over a 5-day period resulted in a response in 5 out of the 6 individuals (Williams, Sudheimer et al. 2018). Advances within the field of depression treatment will likely continue to spread to other neuropsychiatric disorders.

History of rTMS as a treatment for pain.

TMS emerged as a pain treatment emerged in the last two decades, building on findings from electrical stimulation. Early work using motor cortex epidural stimulation for thalamic pain syndrome, as well as central and neuropathic pain in was effective (Tsubokawa, Katayama et al. 1991, Nguyen, Lefaucheur et al. 1999), but highly invasive, and required extensive presurgical planning in order to correctly place the electrodes. One difficulty is identifying the specific region of the cortex to target, in order to minimize surgical procedures. As an addition to functional targeting with neuroimaging, single pulses of TMS were used to identify and map cortical locations (Lefaucheur and Picht 2016). Given the similarities between repeated electrical

and magnetic stimulation, rTMS was explored as a potential mechanism to find responders for the epidural cortical stimulation (Canavero and Bonicalzi 2005, Canavero and Bonicalzi 2007), as even a brief analgesic response to rTMS was associated with higher response rates to surgery and implantation. In the course of these procedures it was discovered that rTMS alone was able to produce long lasting relief from pain (Lefaucheur, Drouot et al. 2004). From these early findings at least 21 studies have been completed examining analgesic effects of rTMS to the motor cortex for chronic pain.

The primary cortical target for pain relief with TMS has been the motor cortex. Most studies have used high frequency stimulation (>5Hz) at amplitudes that are below the resting motor threshold (80 – 95% rMT) (Moisset, de Andrade et al. 2016). These studies have shown TMS-associated pain reduction in healthy controls (Summers, Johnson et al. 2004, Andre-Obadia, Peyron et al. 2006, Nahmias, Debes et al. 2009, Houze, Bradley et al. 2013, Moisset, Goudeau et al. 2015), and individuals with chronic pain (Lefaucheur, Drouot et al. 2006, Passard, Attal et al. 2007, Goto, Saitoh et al. 2008, Lefaucheur, Drouot et al. 2008, Lefaucheur, Jarry et al. 2010, Mhalla, Baudic et al. 2011). Though the mechanism is unclear, these effects can be blocked by naloxone (de Andrade, Mhalla et al. 2011), suggesting that pain relief from motor cortex stimulation is due, at least in part, due to endogenous opioid relief.

Another target is the dorsolateral prefrontal cortex (DLPFC). As discussed previously the DLPFC is heavily involved in modulating the pain experience, including cognitively driven reductions in pain and the placebo response (Wager, Rilling et al. 2004, Schafer, Geuter et al. 2018). In regards to brain stimulation, the DLPFC has undergone research primarily as a treatment of depression, and is currently an effective treatment location for treatment resistant

depression (George, Wassermann et al. 1995, George, Wassermann et al. 1997, George, Lisanby et al. 2010, Blumberger, Vila-Rodriguez et al. 2018).

There is pharmacological evidence that DLPFC stimulation also engages the endogenous opioid system for pain relief, as its effects can also be blocked by naloxone (Taylor, Borckardt et al. 2012, Taylor, Borckardt et al. 2013) though there are conflicting findings (de Andrade, Mhalla et al. 2011). Other work has found that the pain-relieving effects can be blocked by the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine, implicating glutamatergic signaling (Ciampi de Andrade, Mhalla et al. 2014). These findings may need to be revisited in light of the new findings relating ketamine administration with opioid activity (Williams, Heifets et al. 2018), however, it is nevertheless evidence that there is a complex cascade of effects between the cortical stimulation target the analgesic effects. Moving beyond pharmacology, there is limited work examining the functional correlates of rTMS induced analgesia. Changes in the midbrain BOLD signal are apparent after rTMS delivered to the DLPFC, as were increases in DLPFC activity (Taylor, Borckardt et al. 2013). Though these early results are promising, the ideal target for stimulation to attenuate pain remains unknown. By optimizing the target, we may be able to more effectively modulate pain, in both healthy individuals from their first injury and for those currently suffering from chronic pain.

Specific Aims

In order to address the need for a non-pharmacological, brain-based treatment for pain I have constructed a set of interconnected experimental aims:

Aim 1: Demonstrate causal effects of DLPFC stimulation on connected regions. I will report findings from a research project that combines neuroimaging with TMS stimulation at the

DLPFC. In contrast to motor cortex stimulation which has readily accessible behavioral readouts, no such effects are available for DLPFC stimulation. Thus, the first step is a proof-of-principle study. This study will test the hypothesis that DLPFC stimulation, compared to a well-matched control condition, will lead to greater activity within the brain in *a priori* defined regions, based on the neural circuitry associated with the DLPFC target.

Aim 2: Evaluating two stimulation targets for analgesia in healthy controls. This experiment will explore two unique stimulation strategies, compared to a sham intervention. With the first strategy, we will examine if iTBS targeted to the DLPFC can recapitulate the analgesic effects of 10Hz stimulation from prior studies. The second strategy, using cTBS targeted to the medial prefrontal cortex, is a novel approach that is supported by evidence linking the MPFC to pain, but has not yet been evaluated for analgesic effects. Combined neuroimaging, and behavioral measures will be used to test the hypothesis that active, but not sham stimulation leads to an attenuation in pain related brain activity, as well as behavioral measures.

Aim 3: Determine how pain processing differs from healthy controls in population with chronic pain. I will report findings from a study in which pain was delivered in the fMRI environment in order to uncover differences in functional processing of pain between a control population and individuals with chronic pain. Here we will test the hypothesis that pain related activity will be elevated in individuals with chronic pain, which has important implications for the development of a brain-based treatment for those individuals.

This dissertation will conclude with a discussion of these findings, while also reporting pilot data from an rTMS study evaluating pain relief in a population with chronic pain. In this way, the

effects found in healthy controls can be explored in the population of interest – that is – individuals with chronic pain for which a non-pharmacological approach is so urgently needed.

Chapter 2 Proof of Principle: Understanding TMS With Single Pulse Interleaved TMS/fMRI

Introduction

In developing a brain-based treatment for pain, it is first necessary to show proof of the principle that transcranial magnetic stimulation (TMS) has effects that depend on the connectivity of the brain. Due the principles of magnetic field decay, a TMS pulses can only directly have action at the cortical surface, with deep structures out of reach. Given the depth of multiple pain processing regions, this limitation appears substantial, however, there is an important caveat. When TMS activates cortical neurons, these in turn project to distant and deeper locations. In this way this noninvasive tool, which can increase or decrease cortical excitability, can target entire circuits rather than just single cortical sites. There is now substantial evidence using a variety of neuroimaging measures that TMS has action at a distance, and is not rendered ineffective by magnetic field decay.

Previous studies using positron emission tomography (PET), have demonstrated that TMS to the motor or prefrontal cortex can modulate dopamine binding in monosynaptically connected, subcortical areas (Strafella, Paus et al. 2001, Strafella, Paus et al. 2003, Cho and Strafella 2009). PET however, requires the use of a radioligand and has limited temporal and spatial resolution. Another approach to examine the causal effects of TMS on cortical-subcortical circuits is interleaved TMS/fMRI. By applying single TMS pulses between the acquisition of functional volumes, it is possible to measure the brief, transient activation via changes in the BOLD signal

in the cortical areas beneath the coil and in subcortical afferents in the basal ganglia (Bohning, Shastri et al. 1999, Bohning, Shastri et al. 2000, Baudewig, Siebner et al. 2001, Bestmann, Baudewig et al. 2003, Bohning, Shastri et al. 2003). As with the PET studies, this has been demonstrated in the motor system (Barker, Jalinous et al. 1985, Bohning, Shastri et al. 1998, Bohning, Shastri et al. 1999, Bohning, Shastri et al. 2000, Bestmann, Baudewig et al. 2004, Bestmann, Baudewig et al. 2005) as well as prefrontal cortex (Hanlon, Canterberry et al. 2013, Hanlon, Dowdle et al. 2015, Hanlon, Dowdle et al. 2016). Interleaved TMS/fMRI is a powerful tool, that is able to demonstrate causal responses to single TMS pulses, and thereby demonstrate the effects of targeting specific circuits. However, there are still several important methodological considerations and concerns about this technique.

Developing a well-matched control condition has been one challenge for interleaved TMS/fMRI. Without a well-matched control, it is difficult to disentangle the true response to a single TMS pulses from non-specific effects, including startle or effort not to move. Researchers have explored a number of methods thus far, including positioning the coil at a 90 or 45 degree angle to the scalp (Osaka, Otsuka et al. 2007), stimulation at lower intensities (Leitao, Thielscher et al. 2013), vertex stimulation as a control site (Leitao, Thielscher et al. 2013) and increasing the distance between the coil and the head (Leitao, Thielscher et al. 2015). While these techniques are all reasonable approximations, there are a number of limitations to consider. For example, positioning the coil at an angle alters the sensation the subject feels on the scalp, and may still allow a substantial portion of the magnetic field to reach the cortex. (Loo, Taylor et al. 2000, Lisanby, Gutman et al. 2001). Reducing the intensity of the stimulation, even by as much as 40%, can still lead to changes in cortical excitability (Kujirai, Caramia et al. 1993, Di Lazzaro, Oliviero et al. 2004) and the reduction in the sensation and loudness of TMS can be noted by the

participant. Vertex stimulation, which retains the TMS sensation and loudness, is promising, but recent work shows it may result in widespread deactivations across brain networks (Jung, Bungert et al. 2016). Stimulation at any cortical site is likely to have an effect, as no brain region is truly 'silent' and not part of the connected whole.

The lack of a psychophysically-matched control condition may account for inconsistencies found in the literature regarding the effects of TMS on cortical and subcortical afferents. For example, in the prefrontal cortex, several studies have demonstrated that BOLD signal is observed near the coil (Nahas, Lomarev et al. 2001, Bestmann, Baudewig et al. 2005, Hanlon, Canterberry et al. 2013). Other studies, however, have failed to find a difference in BOLD signal in the cortical area under the TMS coil, despite observing modulation in cortical and subcortical afferents (Baudewig, Siebner et al. 2001, Kemna and Gembris 2003, Hanlon, Dowdle et al. 2016). Furthermore, while a few previous studies have demonstrated an intensity-dependent effect of TMS on the BOLD signal (Bohning, Shastri et al. 1999, Nahas, Lomarev et al. 2001), these studies did not control for many of the effects of stimulation, and only a limited range of intensities were explored. The development and evaluation of a control condition that incorporates as many of the sensory aspects, but effectively prevents the entry of the magnetic field may resolve some of these disparate findings. Interest in prefrontal areas, specifically the left dorsolateral prefrontal cortex (LDLPFC), stems from its importance as a clinical target. At present, the LDLPFC is the FDA approved treatment site for depression (George, Lisanby et al. 2010) and is being explored as a treatment for other psychiatric conditions, including addiction (Barr, Farzan et al. 2011, Bellamoli, Manganotti et al. 2014, Gorelick, Zangen et al. 2014, Grall-Bronnec and Sauvaget 2014) and pain (Lefaucheur, Antal et al. 2008, Galhardoni, Correia et al. 2015, Moisset, de Andrade et al. 2016).

In order to demonstrate that TMS is causally effecting distance cortical sites we have evaluated a control condition in which we increased the coil to cortex distance using 3 cm of firm padding. This preserved many of the sensory aspects of the procedure, while considerably reducing the magnetic field. Additionally, we varied the TMS machine output to evaluate potential intensity-dependent effects of TMS on the evoked BOLD signal. This experimental design was used to test the hypothesis that compared to a control, active TMS would selectively elevate the BOLD signal in the left DLPFC (the site of highest clinical relevance) and subcortical targets.

Materials and Methods.

Participants and Procedure.

Using word of mouth and digital advertising we recruited twenty healthy individuals from the local community (Table 1). Following written informed consent (approved by the Medical University of South Carolina Institutional Review Board), we invited all eligible participants to the Center for Biomedical Imaging for the experimental visit. Exclusion criteria included a history of seizures, head trauma or a loss of consciousness greater than 15 minutes, history of brain surgery or lesions, use of medications that lower seizure threshold, failure to meet typical MRI safety guidelines, current unstable medical illness or past 6-month illicit drug use.

Table 2.1 Demographics

Table 1. Demographics	
N	20 (14 females)
Age	26.8±4.9
Race	17 Caucasian, 3 AA
Education	17.9±3.0 years
Resting Motor Threshold	68.3±8.6

Upon arrival, we identified the target for the TMS stimulation (left dorsolateral prefrontal cortex (DLPFC), Beam F3 method (Beam, Borckardt et al. 2009)) and marked on a Lycra® swim cap (Water Gear Inc., Pismo Beach CA, 0.5mm thickness) which remained in place for the duration of the visit. Resting motor threshold (rMT) was then determined using the same Magstim figure 8 coil and a Magstim SuperRapid capacitor (Magstim Inc.) that were subsequently used for the interleaved TMS procedure. rMT was found by modulating the stimulator output until a value resulted in a thumb twitch in 5 out of 10 trials (Rossini, Barker et al. 1994). The average rMT was 68.3% of the machine output (±8.6%).

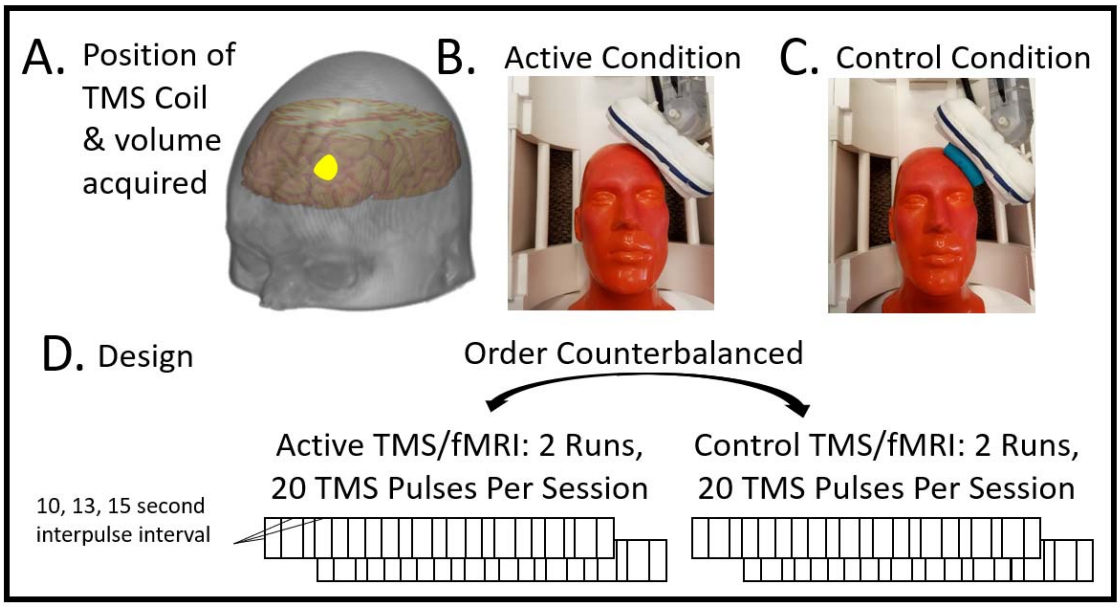

Figure 2.1 Basic Study Set-Up and Design. A: Approximate scalp location of DLPFC, as defined by EEG 10-20 coordinate F3. Brain section within head image shows approximate extent of data collected. B: An example of the coil position for the active stimulation condition. C: An example of the coil position for the sham control condition in which 3cm of open-cell reticulated foam padding was placed between the coil and F3. D: Task design for interleaved TMS/fMRI. Active and Control Stimulation were counterbalanced. In each case, a pre-randomized interpulse interval was used and the TMS machine output (intensity) was varied throughout the acquisition.

Interleaved TMS Delivery.

Participants were then positioned supine on the bed of the MRI scanner with their head placed securely in a 12 channel head coil (RAPID Biomedical [Rimpar, Germany]) with a built in TMS coil mount (Bohning, Denslow et al. 2003). We then placed compressible padding on either side of the head to reduce motion during MRI acquisition. For the "active" stimulation condition, we placed the TMS coil over the marked location on the swim cap (Figure 2.1A, B). For the "control" stimulation condition, we placed 3cm of firmly compressed open-cell reticulated foam padding between the TMS coil and the head (Figure 2.1C). Increasing the distance, d, dramatically weakens the strength of the magnetic field, B, as approximated by the function, $B(d) = 1.05e^{-0.036d}$ (George, Bohning et al. 2007), adapted from (Bohning, Pecheny et al. 1997). With this equation, a 3cm increase in coil to cortex distance results in a 66% reduction in the strength of the magnetic field. This value is in agreement with prior work showing that each millimeter of added distance from the scalp is equivalent to a 3% reduction in stimulator output (Stokes, Chambers et al. 2005, Stokes, Chambers et al. 2007), though this linear approximation is derived from more typical distances (i.e. 10 mm) (Stokes, Barker et al. 2013). Each participant received 4 interleaved TMS/fMRI runs (two active and two control, 20 TMS pulses per run, presented in a counterbalanced order, Figure 2.1D). The order of the interpulse interval was randomized prior to study initiation to be 10, 13 or 15 seconds. Onsets were set as a list in E-Prime software (Psychology Software Tools, Pittsburgh, PA), which counted each TR, using TTL pulses produced by the scanner. When the appropriate TR was reached, a TTL pulse was sent to the MagStim TMS device, triggering each TMS pulse during a gap (ie. after 900ms) between

volumes (see MRI acquisition). Correct stimulation timing was confirmed by the absence of TMS firing artifacts during volume acquisition.

When switching from active to control stimulation, we told participants that we were validating the position of the TMS coil. For all runs, the lead author varied the stimulator output (randomized prior to study initiation) by hand to values between 90 to 120% (10% steps) of each participant's rMT. Each change was made during the 10 -15 second gap between TMS pulses. Correct timing was ensured through the use of a checklist and stopwatch. For the primary study, we evaluated the integrity of the control condition by asking each participant to state whether a given run felt more or less painful than the proceeding run. Of the individuals stating that they perceived a difference, 2 individuals reported that the control runs were slightly more painful and 3 reported that the active runs were more painful.

Sub-study evaluating the sensory aspects of active and control stimulation. To better quantify the sensory aspects of the stimulation**,** ten participants were invited back in for a repeat visit (4 male, Age:26.1±3.5, rMT: 69.9±8.8. Four sessions of TMS were performed, 2 active and 2 control, with the order fully counterbalanced. Each session of TMS contained 10 pulses, with the amplitude varied throughout the acquisition, as in the primary experiment above. After each session, the participants were asked a series of questions on a scale of 0 to 10: "Overall, how painful was the last session?", "Overall how unpleasant was the last session?", "Overall, how intense and strong were the TMS pulses in the last session?" and "Overall, how loud were the TMS pulses during the last session?". For the 2nd and subsequent TMS runs, they were also asked "Did this TMS session feel the same or different from the previous session?" and asked to rate their confidence on that decision, again on a scale of 0 to 10.

After the end of the last session, each participant was told that the purpose of this study was to determine if they received an active stimulation or a stimulation condition wherein the coil was positioned away from the head using padding (i.e. control stimulation). They were then asked to guess, for each session, whether they thought they received active or control stimulation. Each sensory aspect (pain, unpleasantness, intensity and loudness) was analyzed in SPSS using mixed modeling with condition (real vs sham), time, and the condition*time interaction as predictors. Individual subject intercepts and time slopes were entered as random effects in the model. The model employed restricted maximum likelihood estimation (REML) and the covariance structure was specified as "unstructured". The collective accuracy of each participants guess was entered into a contingency analysis using Fisher's exact test to determine performance compared to chance. Confidence for correct guesses and incorrect guesses was averaged.

MRI acquisition.

A Siemens 3T TIM trio scanner (Siemens, Erlangen, Germany) and 12 channel head coil was used for all imaging. For both active and control conditions anatomical images (T1 weighted, MPRAGE, 1mm isotropic, 192 slices per slab, TR 1620 ms TE 2.26 ms), a field map (3.4x3.4x3.0 mm, 43 slices, TE_1 4.6 ms, TE_2 7.06 ms) and a whole brain T2* weighted anatomical image (3.4x3.4x1.8 mm, 63 slices, TR 3470ms, TE 23 ms) were acquired before the interleaved TMS/fMRI procedure (3.4x3.4x4.0mm, 16 slices, TE 23 ms, TR 1000ms, Flip Angle 60 degrees). The 23 ms echo time was used to reduce susceptibility artifacts, as well as to improve comparability to prior work (Shitara, Shinozaki et al. 2011, Hanlon, Canterberry et al. 2013, Shitara, Shinozaki et al. 2013, Hanlon, Dowdle et al. 2016), which used similar values. The short

TR in the interleaved TMS/fMRI acquisition was used to better capture the TMS response, but required reducing the number of slices. These limited field of view (FOV) data were acquired with a negative pitch from the AC-PC line (Figure 2.1A). All 1000ms TR consisted of 900ms of volume acquisition, followed by a 100ms gap, during which the MRI was inactive. Each TMS pulse is triggered to occur at the beginning of this gap, identical to previous work from our group (Bohning, Shastri et al. 1998, Hanlon, Canterberry et al. 2013).

Imaging data preprocessing. SPM12, running in MatLab 2012a (The MathWorks Inc.), was used for data preprocessing. For each participant, the limited FOV T2* images were coregistered to the whole brain T2* image using the mutual information algorithm in *Coreg: Estimate*. Next, field map derived voxel displacement maps were calculated, and coregistered to the whole brain T2* image (*VDM Toolbox*). *Realign and Unwarp*: *Estimate and Reslice* was then used to align volumes across time and reduce image distortion. The high resolution MPRAGE was processed through unified segmentation (*Segment)*, which simultaneously derives tissue masks (used to mask out the skull with *ImCalc*) and the nonlinear deformations required to warp images into standard space. The full brain T2* images from both active and control sessions were realigned to skull stripped anatomical images, and the limited FOV images were kept in register (*Coregister: Estimate)* and nonlinear deformations were applied (*Normalise: Write*). At this point any transient, single slice artifacts were removed from the normalized data using the default settings of 3dDespike from AFNI (average percent of big edits: 0.44±0.19, for temporal signal to noise ratio image see Figure 2.2). Data were smoothed using an 8mm FWHM Gaussian kernel (*Smooth)*. Estimated motion parameters were examined and no subject exceeded the movement threshold of one voxel.

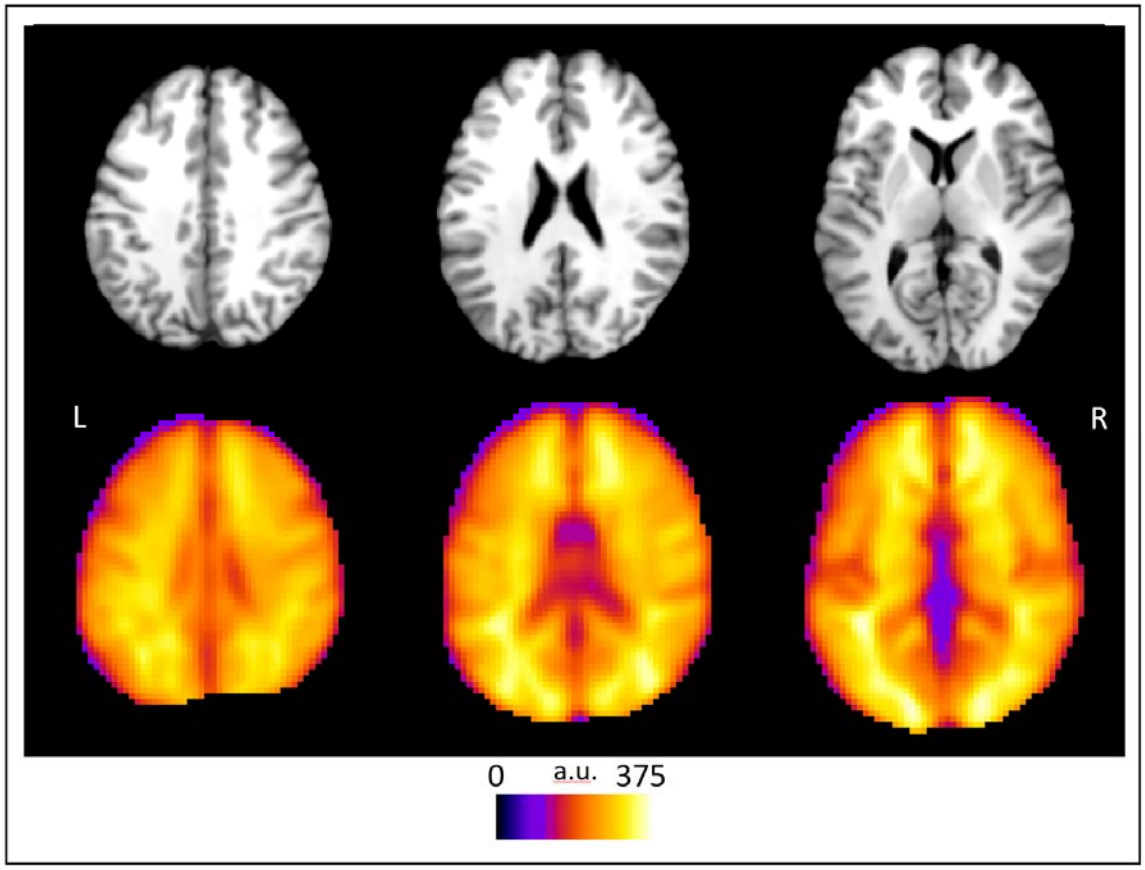

Figure 2.2 Temporal Signal to Noise Image. This figure shows axial slices through the brain with values of the temporal signal to noise ratio (tSNR). The tSNR is calculated by dividing the mean of each voxels timeseries by the standard deviation. Here, we highlight that there is not an association of signal loss or increased noise on the left side of the image (the location of the TMS coil) compared with the right.

Statistical analyses.

The preprocessed data was used for within-subject, general linear modeling. Each level of TMS machine output was modeled separately as a series of instantaneous events, convolved with the canonical double gamma hemodynamic response model provided with SPM12. For nuisance regressors, we used a set of expanded motion parameters. These included the 6 rigid

body parameters from SPM's Realign and Unwarp, their derivatives and the square of the original and derivatives (Satterthwaite, Elliott et al. 2013). To remove low frequency drift, we used a high pass filter of 45 seconds and applied SPM12's FAST model to account for autocorrelations. To determine if a linear intensity dependent effect could be found, a separate subject-level model was used. This model was identical to the above, except all TMS onsets were entered as a single vector, with 1st order parametric modulation corresponding to the intensity entered as a second column in the design matrix. This modulates the height of each TMS event by the intensity, and was used to determine if a linear effect is present. **Whole volume analysis**. For group level analysis, a factorial design was used (active vs control X Machine Output) which included eight contrast maps per subject (4 levels of machine output for both active and control). For intensity dependent effects, the subject-specific contrasts corresponding to the positive linear effect during active and control stimulation were entered into a two sample t-test. **Regions of Interest.** A region of interest (ROI) analysis was also performed using anatomically defined ROIs motivated by previous literature. The preprocessed data was converted to units of percent signal change using the CONN toolbox, version 16.b (Whitfield-Gabrieli and Nieto-Castanon 2012), for the toolbox: www.nitrc.org/projects/conn). Processing was limited to identical high pass filtering and movement parameter regression. The following ROIs were generated from the WFU Pick Atlas (Tzourio-Mazoyer, Landeau et al. 2002) and Oxford Thalamic connectivity atlas (Behrens, Woolrich et al. 2003): caudate, putamen, left middle frontal gyrus (LMFG, area under the coil) RMFG, anterior cingulate cortex (ACC), superior temporal cortex (auditory regions, positive control region), globus pallidus and prefrontal zone of the thalamus (Figure 2.4). Except for the left and right MFG, we chose bilateral ROIs to best capture ipsi- and contralateral projections. Time course extraction was completed using

MarsBar (Brett, Anton et al. 2002). Time courses were exported to Excel (Office 365, Microsoft) and peak activation was extracted by finding the maximum value between 3 and 8 seconds following each TMS pulse. These peaks were averaged (by machine output), yielding a single value for each level of output, ROI and subject. These values were then used in all subsequent statistical analyses, which were performed in GraphPad Prism 7.02 (La Jolla, CA, USA).

Results

Effect of active versus control TMS on whole brain BOLD signal.

(Figure 2.3**)** Single pulse TMS to the left DLPFC led to elevated BOLD signal in multiple brain regions including the left and right middle frontal gyrus, the bilateral insula, thalamus, superior temporal cortices (auditory cortex), and anterior cingulate (Table 2.2, Figure 2.3A, voxelwise $p < 0.05$, FWE corrected). Control TMS to the left DLPFC, however, also led to elevated BOLD signal in many of these regions (Figure 2.3B, voxelwise $p<0.05$, FWE corrected).

Relative to the control condition, active TMS led to significantly greater BOLD signal in the caudate & thalamus (Cluster 1, $p < 0.05$, FWE corrected) as well as the anterior cingulate cortices (Cluster 2: $p = 0.025$, uncorrected) (Figure 2.3C, cluster forming threshold $p< 0.01$). There were no areas in which control TMS resulted in greater activation (Table 2.2).

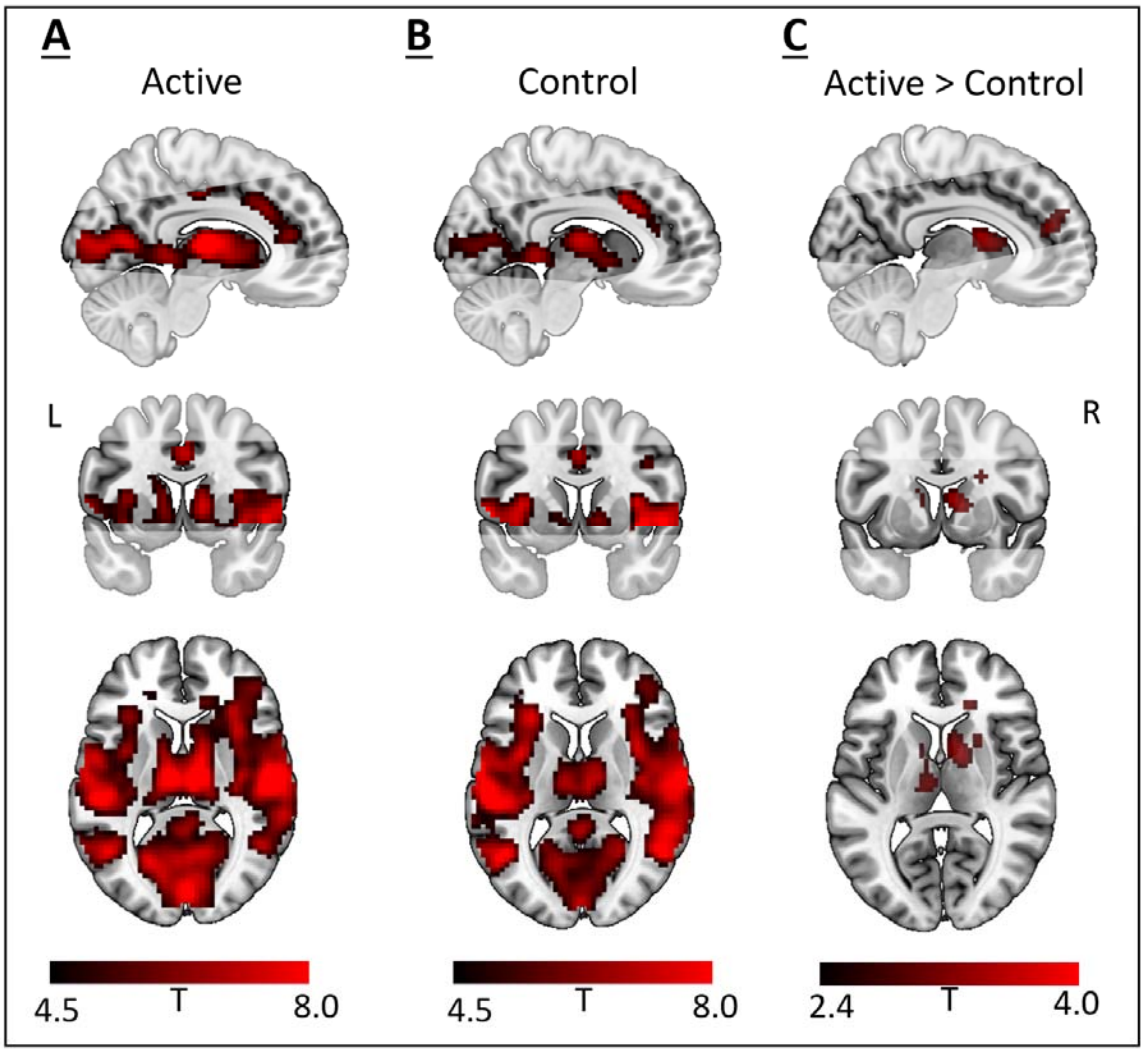

Figure 2.3 Results from Interleaved TMS/fMRI. For coronal and axial slices the left side of the brain is on the left. Fading of dorsal and inferior aspects show areas not available for analysis due to limited field of view. A shows the group statistical parametric map in response to active stimulation, combined across all TMS machine output levels (threshold: voxelwise $p<0.05$, FWE). Panel B shows the same map for control stimulation (threshold: voxelwise $p<0.05$ FWE) which is very similar. Panel C shows the positive effect of Active compared to Control stimulation (cluster threshold: $p<0.01$, uncorrected), in which only frontostriatal afferents are significantly more active in active as compared to control stimulation.

Table 2.2 Results from analyses of active and control stimulation.

Cluster Statistics			Cluster Locations	Peak Location (MNI)		
$p_{FWE\text{-}corr}$	Number of voxels	$p_{uncorrected}$		x	y	z
Active Stimulation Only						
<0.000	9222	<0.000	B Sup. Temporal, B Mid. Frontal, B Caudate, B Putamen, B Insula	63	-22	17
				-60	-28	17
				9	-16	8
0.001	30	0.012	R Cuneus, R Precuneus, R Angular	33	-52	38
				18	-61	35
				27	-58	35
<0.000	122	<0.000	L Mid. Frontal	-33	47	14
				-24	38	20
				-18	38	5
0.016	4	0.316	R Precuneus, R Mid Cingulate	15	-46	38
0.032	1	0.633	n/a	24	8	26
Control Stimulation Only						
<0.000	7059	<0.000	B Sup. Temporal, B Insula, B Caudate, B Thalamus, B Putamen	-63	-28	20
				63	-25	20
				-63	-37	23
<0.0000	431	<0.000	B Anterior Cingulate, B Mid Cingulate, B Sup. Med. Frontal	6	14	35
				3	23	32
				3	38	14
<0.000	45	0.003	B Mid Cingulate	3	-22	29
				3	-31	26
0.002	17	0.049	R Mid. Frontal	33	47	32
0.025	2	0.484	L Pallidum	-18	-4	-7
0.025	2	0.484	L Mid. Cingulate	-12	-28	41
0.025	2	0.484	R Mid Cingulate	15	-31	38
0.025	2	0.484	R Mid. Frontal	27	44	20
Active greater than Control						
0.254	168	0.025	R Anterior Cingulate, R Sup. Frontal, R Sup. Med., R Mid. Frontal,	15	47	23
				15	32	11
				27	53	26
0.048	304	0.004	L Thalamus, B Caudate	6	2	14
				33	11	17
				24	26	26

Active and Sham only stimulation peaks are reported from the voxelwise $p < 0.05$ (FWE corrected) threshold. For the Active greater than Sham comparison, peaks are reported for a threshold of $p < 0.01$, uncorrected, with a cluster size threshold of 100.

Whole brain intensity dependent effects

There were no areas that showed a significant linear relationship between machine output and the height of the hemodynamic response in either active or control stimulation.

Effects of active versus control TMS in predefined regions of interest.

(Figure 2.4**)** Active TMS led to a significantly greater BOLD signal in the caudate ($F_{1, 19} = 6.036$, $p=0.0238$, active PSC: 0.616±0.057, control PSC: 0.544±0.047), the anterior cingulate cortex ($F_{1, 19} = 4.727$, $p=0.0425$, active PSC: 0.359±0.019, control PSC: 0.313±0.019). There was no significant difference between active versus control TMS in the other regions of interest investigated (left middle frontal gyrus, right middle frontal gyrus, putamen, pallidum, thalamus). There was also no significant difference between BOLD signal in the left auditory cortex, as defined by a region of interest in the superior temporal cortex (positive control region). There were no regions in which control TMS led to greater BOLD signal compared to active stimulation.

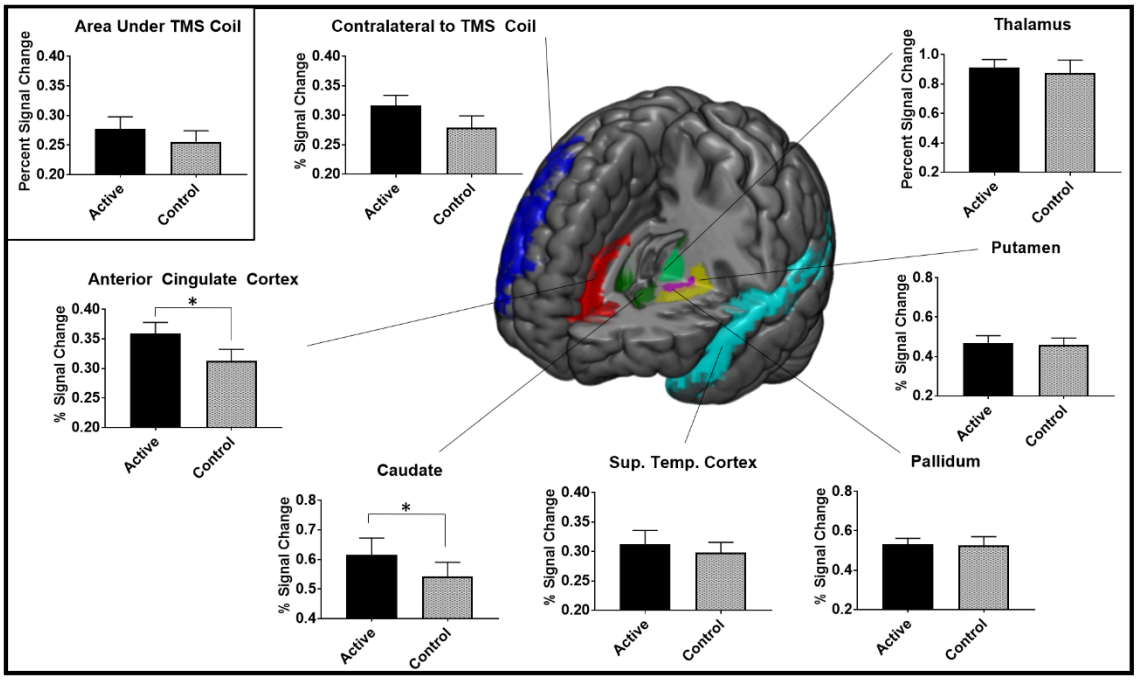

Figure 2.4 Peak Responses in Regions of Interest. The center whole brain shows the ROIs used. All ROIs are bilateral, with the exception of the right and left middle frontal gyrus (inset) ROIs. Both the anterior cingulate cortex and the caudate show a significant main effect of treatment (active vs control stimulation).

Intensity dependent effects in predefined regions of interest

Peak responses from connected ROIs (RMFG, ACC, caudate, superior temporal cortex, putamen, pallidum, thalamus) during stimulation were entered into a linear regression analysis in GraphPad Prism to determine if there was a relationship between machine output and peak response. There was no significant linear relationship between stimulator output in either active or control stimulation.

Sensory Aspects from Sub-study

There was no significant difference between active and sham in pain ($p=0.220$), unpleasantness ($p=0.624$), intensity ($p=0.347$) or loudness ($p =0.451$) (See Figure 2.5). Overall accuracy of guesses was 67.5%. Guessing performance was not significantly better than chance according to Fisher's exact test ($p=0.1086$). The average confidence was 7.33 when participants correctly identified that a session was different, and 7.44 for incorrect identifications.

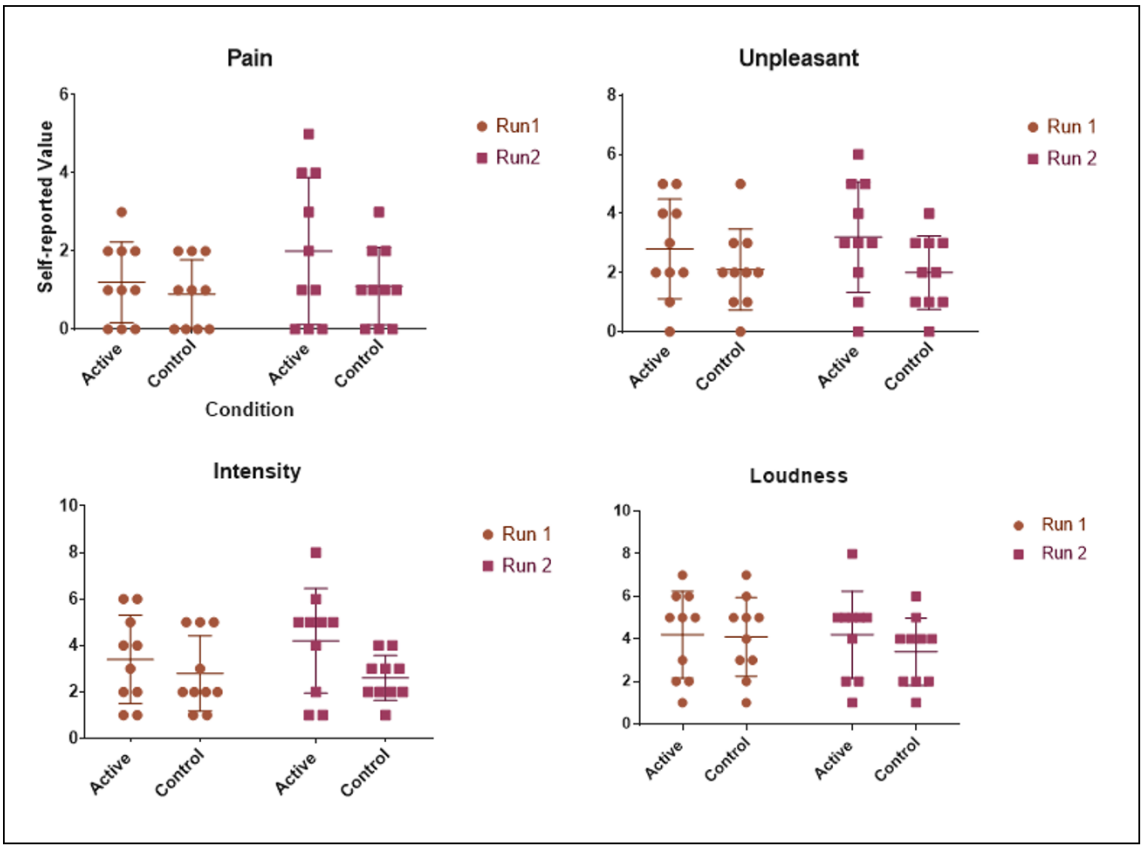

Figure 2.5 Ratings of Active and Sham TMS in Sub-Study. Participants were aske to rate the painfulness, unpleasantness, intensity and loudness of each session of interleaved TMS/fMRI. There were no significant differences between ratings, suggesting that the control condition was well-matched on these measures.

Discussion

Summary

This study presents a direct examination of BOLD signal changes from interleaved TMS/fMRI to the LDLPFC compared to a matched control condition that dramatically reduces the entry of the magnetic field. Consistent with previous studies these data demonstrate that DLPFC TMS leads to elevated BOLD signal in the cortex in the vicinity of the coil as well as in cortical and subcortical afferents, including the striatum and thalamus. As an extension of those studies however, this study demonstrates that the control stimulation condition also produces large BOLD signal in many of the same regions. Active prefrontal F3 TMS evoked significantly more BOLD signal than did control TMS in three specific regions, the caudate, the cingulate, and the thalamus – all of which would be predicted by the basic neuroanatomy. The large BOLD response evoked by the control condition in this experiment underscores the need for routine use of control conditions in interleaved TMS/fMRI literature. These data also suggest that our interpretation of data from previous and future studies using interleaved TMS/fMRI should be tempered by the possibility that many of the TMS-evoked changes in BOLD signal may be indirectly related to sensory and attentional aspects of the TMS pulse. This is of particular importance in drawing conclusions about areas that are not expected on the basis of anatomy to be activated in a direct manner by a given TMS target.

Similarities Between Active and Control Stimulation

As stated above, the data from this study largely replicate observed patterns that have been demonstrated previously, but, importantly the present study reveals that this pattern is largely

preserved when the coil is moved 3cm away from the scalp. At this distance, the magnetic field strength has markedly decayed, suggesting that much of widespread activation in traditional interleaved TMS/MRI protocols is due to experimental factors that are indirectly (rather than directly) related to the magnetic-field. The observed similarity between the active and the control condition is likely due to several factors. Within auditory processing regions similar activation is likely due to the startling nature of each pulse, as it is accompanied by a loud pronounced click. The volume of each click is magnified due to the acoustics of the MRI bore and the force on the TMS coil increasing due to the static magnetic field. These increased forces also cause the physical sensation of single TMS pulses (the physical feeling of a 'tap') to be greater than when compared to TMS delivered outside of the MRI environment. Additionally, we ask that participants remain still in response to this startling stimulus, which requires motor control. Together these factors contribute to widespread brain activation that is time-locked to the TMS pulse, but not necessarily related to the circuit that is targeted. Capitalizing on distance, as was done in previous work targeting the intraparietal sulcus (Leitao, Thielscher et al. 2015), appears to be a reliable way to control for the non-specific effects of TMS stimulation. The addition of 3cm of open-cell reticulated foam in the present study controls for the volume of the click, the pressure on the scalp, the angle and position of the physical sensation on the head, yet adds enough distance that the strength of the magnetic field is insufficient to depolarize cortical neurons. Our findings further support the literature highlighting the importance of controlling for the non-specific effects of TMS, which are invariably time-locked to each pulse.

Active Stimulation Associated with Increased Signal

In the present work, the consistent difference observed between active and control TMS in the caudate (a striatal region monosynaptically connected to the DLPFC) buttresses the long-standing statements that TMS applied to the cortex can induce a change in activity in striatal targets. This TMS evoked change in the caudate has been demonstrated previously using multiple modalities including BOLD signal (Hanlon, Canterberry et al. 2013) and dopamine binding (Strafella, Paus et al. 2001). The caudate and DLPFC have high functional connectivity (Choi, Yeo et al. 2012) and correlated activity as examined by large scale meta-analyses (Pauli, O'Reilly et al. 2016). Another region which was significantly more active during active versus control TMS to the DLPFC was the ACC. As the ACC projects to neighboring regions of the caudate when compared to the DLPFC (Haber and Knutson 2010), its increased activity during active stimulation could reflect additional regulatory processes. The final region that showed a difference between active and control stimulation is the prefrontal zone of the thalamus. This region represents the targets of striatal projections prior to looping back to cortical locations (Middleton and Strick 2000). Notably, this effect in the thalamus was observed in the whole brain, voxelwise analysis but not the region of interest analysis, suggesting that the thalamic effects are not as robust as the effects in other ROIs. Finally, it is important to note that no difference ($p=0.43$, percent signal change: active 0.31±.02, control 0.30±0.02) was found in the ROI that was used as a positive control region, the superior temporal ROI.

Dose Response

The present study failed to find intensity-dependent effects on BOLD signal in any region examined in active or control stimulation. When linear regression was performed in an

exploratory fashion, on a dataset that combined active and control stimulation, only the superior temporal cortex ROI showed a significant linear relationship with stimulator output ($p = 0.0442$). This likely reflects the relatively subtle increase in loudness related to increases in stimulation output, which has been previously been reported (Hanakawa, Mima et al. 2009). In general, the intensity dependent effects of TMS pulses on evoked BOLD signal have been inconsistent in previous literature. The present findings differ from previous work which found that TMS at higher intensities leads to greater activity when delivered over the motor cortex (Bohning, Shastri et al. 1999) and in the left DLPFC (Nahas, Lomarev et al. 2001). These studies differ in several ways from the present study, in that the stimulation profiles, analysis methods and levels of stimulator output differed. In the present work, TMS was delivered at < 0.1Hz, compared to the long (18 or 21 second) 1Hz blocks used in the earlier studies. The early studies also compared these blocks to similar length periods of rest, and used machine output as low as 80%. Together these factors make direct comparisons difficult, though future studies will likely need to use even greater ranges of stimulator output to capture a dose-response curve.

Limitations

In order to execute this study with high temporal resolution functional MRI in a time period which did not overburden the participants, we had to make several compromises to the design which limit its generalizability. One limitation in interpreting these results is that the acquired data were restricted to 16 slices that centered around the AC-PC line, as these slices contain the majority of the cortical and subcortical afferents from the DLPFC. The acquisition protocol did not capture the cerebellum or complete volumes of the dorsal aspects of the cortex (including dorsal parietal, primary sensory, motor, and premotor cortices). These areas should be explored

in future work, as functional connectivity may underlie the clinical effects of TMS (Fox, Buckner et al. 2012) and can involve large scale, whole-brain networks (Fox, Buckner et al. 2014). Additionally, the absence of dose-effects may reflect the range of intensities that were chosen, though previous studies through have also failed to find intensity dependent effects of TMS on evoked BOLD signal under the coil using intensities as high as 150% rMT (Kemna and Gembris 2003). Alternatively, detecting these dose-effects may requires more than 40 TMS pulses divided into the 4 different intensities used in the present study. The total number of pulses used in this study is below the number in early work, which used more rapid stimulation profiles, from 0.83 to 10Hz (Bohning, Shastri et al. 1998, Bohning, Shastri et al. 1999, Bohning, Shastri et al. 2000, Bestmann, Baudewig et al. 2003, Kemna and Gembris 2003, Bestmann, Baudewig et al. 2004, Blankenburg, Ruff et al. 2008). A future study that delivers more pulses, with a wider range of machine output may be needed to further determine how TMS output leads to changes in the BOLD response. Finally, in retrospect it would have been very valuable to ask the original sample of 20 participants to provide a more comprehensive, quantitative evaluation of the active versus control condition. The follow-up study on a repeated sample of 10 of these individuals provides evidence that the control condition was well matched in the sensory domain, however, future work should improve on this design and evaluate further aspects, such as attention or anticipation.

Conclusions

This study sought to determine if a difference could be found between an active stimulation condition and a control condition which included an additional 3cm displacement of the TMS coil from the participant's scalp – effectively eliminating direct magnetic field effects on cortical

excitability. The data reveal strong similarities in evoked BOLD response by active TMS and control TMS. There were however significant differences in the TMS-evoked BOLD signal in the caudate, thalamus, and the cingulate cortex –areas which are strongly predicted by previous research in this area as well as their neuroanatomical connectivity to frontal-striatal-thalamic loops. These differences highlight that TMS can effectively reach distant targets within the brain, while the similarity in activation patterns seen under conventional analyses highlights the critical importance of controlling for the non TMS-specific effects.

Acknowledgements

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Chapter 3 Evaluating TMS as a Tool to Reduce Acute Pain in Healthy Controls

Introduction

Pain is associated with enormous financial costs in the United States, estimated as high as $635 billion (Gaskin and Richard 2012). Currently, prescription opiates are the standard treatment for acute pain (Wu and Raja 2011), however the chronic use of opiates is a rapidly escalating crisis in the United States. Over 4.3 million Americans are dependent on opiate analgesics (SAMHSA 2015). An escalating rate of opiate overdose deaths (Center for Disease Control and Prevention 2015), and a resurgence of intravenous heroin use leading to total societal cost exceeding $55 billion (Birnbaum, White et al. 2011). Among those who misuse opioids, 80 to 90% initiated after having a legitimate prescription (Barth, Maria et al. 2013, Shei, Rice et al. 2015) and 81% endorse pain as their reason for non-medical prescription opioid use (NMPOU) (Barth, Maria et al. 2013). There is an urgent need to develop new treatments for acute and chronic pain.

As discussed in Chapter 1, the pain experience is constructed in the brain (Figure 3.1) and involves a wide range of cortical and subcortical structures. The most commonly activated structures in healthy controls experiencing acute pain in the fMRI environment are the insula, anterior cingulate and thalamus (Apkarian, Bushnell et al. 2005). Typically when evaluating pain processing in the fMRI environment these response to pain are divided into early and late phases (Becerra, Breiter et al. 1999, Wager, Rilling et al. 2004, Price, Craggs et al. 2007, Eippert, Bingel et al. 2009, Upadhyay, Pendse et al. 2010). The early phase is time locked to the stimulus and is thought to reflect the encoding information such as location, while the late phase is

delayed and appears related to the evaluation of the pain and context (Taylor and Fragopanagos 2005, Kong, White et al. 2006, Price, Craggs et al. 2007, Moulton, Pendse et al. 2012).

One method which can target these specific regions related to pain process is noninvasive repetitive transcranial magnetic stimulation (rTMS). rTMS refers to delivering TMS pulses, which pass directly through the scalp to activate the underlying cortex (see Chapter 1), in a specific frequency or repeated pattern. Work from the motor cortex has showing that rTMS has the capability to induce effects that mimic long-term potentiation (LTP) or long-term depression (LTD) in target cortical regions. The directionality of the effects is frequency dependent, in that higher frequencies (>5Hz) tend to lead to LTP-like effects (Berardelli, Inghilleri et al. 1998, Maeda, Keenan et al. 2000), whereas lower frequencies (1Hz) tend to promote LTD-like effects (Chen, Classen et al. 1997, Maeda, Keenan et al. 2000, Gerschlager, Siebner et al. 2001). Prior work has shown that 10Hz stimulation using repetitive transcranial magnetic stimulation (rTMS) targeted to the left DLPFC is able to attenuate the brain response to acute thermal pain in healthy controls (Taylor, Borckardt et al. 2012, Taylor, Borckardt et al. 2013), and associated with reductions in activity of the anterior cingulate cortex. One difficulty is that these conventional 10Hz protocols take up to 30 minutes. Recently research has been executed with biologically-based, faster acting protocols, known as Theta Burst Stimulation (TBS) (Huang, Edwards et al. 2005). When delivered in brief, 2 second bursts separated by 8 seconds, this method is known as intermittent theta burst (iTBS), and has similar results to 10Hz stimulation (Huang, Edwards et al. 2005, Blumberger, Vila-Rodriguez et al. 2018) in as little as 3 minutes. An alternative method, known as continuous theta burst stimulation (cTBS) takes as little as 40 seconds and recent work from our lab found that stimulation to the medial prefrontal cortex (MPFC) reduced TMS evoked responses in the insula, and other regions of the pain matrix

(Hanlon, Dowdle et al. 2017). This area has yet to be explore as an analgesic treatment site, however it is positioned at the intersections of multiple brain processes (Roy, Shohamy et al. 2012), including reward evaluation (Dunlop, Hanlon et al. 2016) , emotional processing (Etkin, Egner et al. 2011) and fear conditioning (Schiller and Delgado 2010) that are related to pain processing.

Here we report the results of a sham-controlled, single blind experiment designed to evaluate the effectiveness of rTMS in reducing the brain and behavioral responses to pain in a thermal pain task. In this study we evaluated two potential strategies to determine the effectiveness, relative to an active sham, of a single session of either iTBS at the DLPFC or cTBS at the MPFC in reducing acute (early and late phase) pain responses in healthy controls. We hypothesized, on the basis of prior work, that both types of stimulation would result in reduced behavioral responses to pain, with site specific effects on the brain response. Specifically, targeting the DLPFC would lead to reductions in the anterior cingulate cortex, while MPFC stimulation would result in an attenuation of insula reactivity.

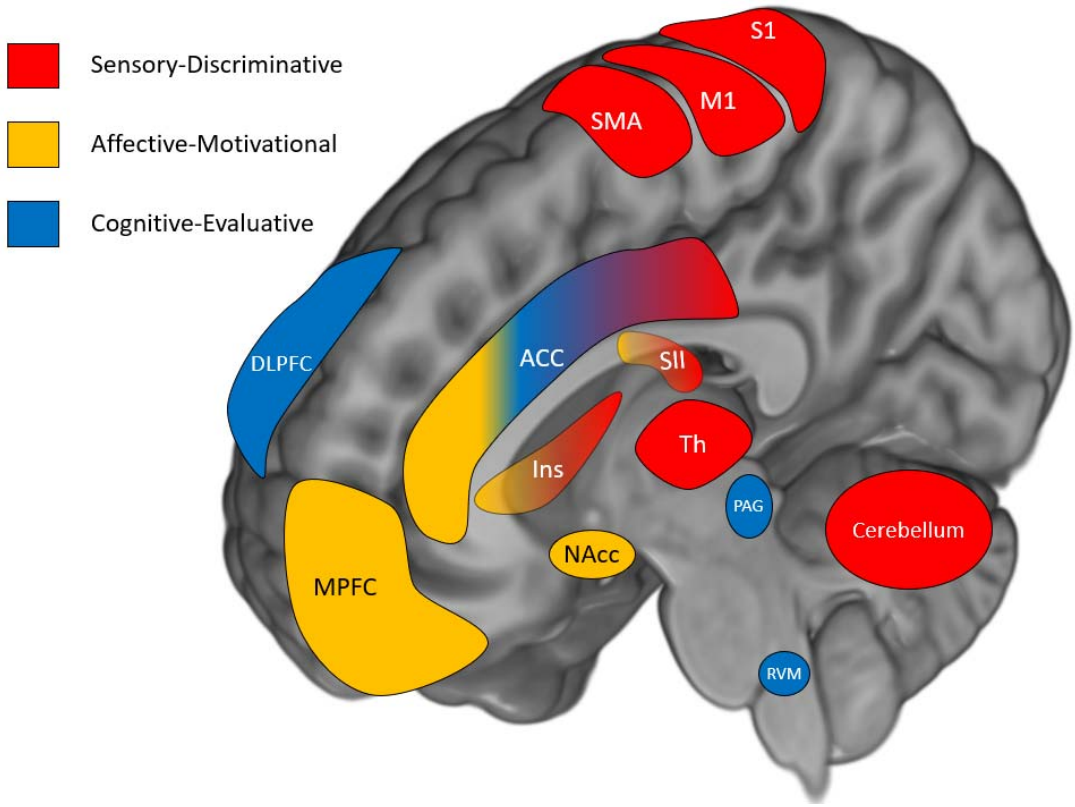

Figure 3.1 Brain regions associated with pain processing. Nociceptive projections ascend from the spine to the thalamus and from there project to multiple areas in the brain. Regions involved in the pain response can be broadly classified into sensory-discriminative (Red) that identify the location and type of pain; affective-motivational areas (Yellow) that deal with emotion processing and motivated responses as well as cognitive-evaluative (Blue) areas that regulate the pain experience. Some areas perform multiple functions, here indicated by blending colors. RVM – Rostral Ventromedial Medulla, APG - Periaqueductal Gray, NAcc - nucleus accumbens, Th – thalamus, Ins - insula, SI - primary sensory cortex, SII – secondary sensory cortex, SMA – supplementary motor area, MI – motor cortex, DLPFC – dorsolateral prefrontal cortex, MPFC – medial prefrontal cortex, ACC – anterior cingulate cortex.

Materials and Methods

Participants and Procedure.

Using word of mouth and digital advertising we recruited a total of 51 participants from the local community. Six of these subjects were excluded from the final analyses, leaving a total of 45 subjects. Reasons for exclusion were metal artifact (n=1), loss of pain sensitivity (n=4) and loss of interest in study (n=1). This study consisted of two experimental visits (Figure 3.2). **Visit 1:** Participants were invited to the Center of Biomedical Imaging. After an explanation of study procedures and an opportunity to ask questions, participants provided written informed consent (approved by the Medical University of South Carolina Institutional Review Board). Following consent, we completed the following questionnaires: Beck Depression Inventory II (BDI-II), Pittsburgh Sleep Quality Index (PSQI), State Trait Anxiety Inventory (STAI), Brief Pain Inventory (BPI), Profile of Mood States (POMS), MINI, Barratt Impulsiveness Survey (BIS) and an questionnaire concerning current pain, stress, discomfort and pain relievers (Pain Questionnaire) and a urine pregnancy screen and urine drug screen. Exclusion criteria included a history of seizures, head trauma or a loss of consciousness greater than 15 minutes, history of brain surgery or lesions, use of medications that lower seizure threshold, failure to meet typical MRI safety guidelines, current unstable medical illness or current illicit drug use.

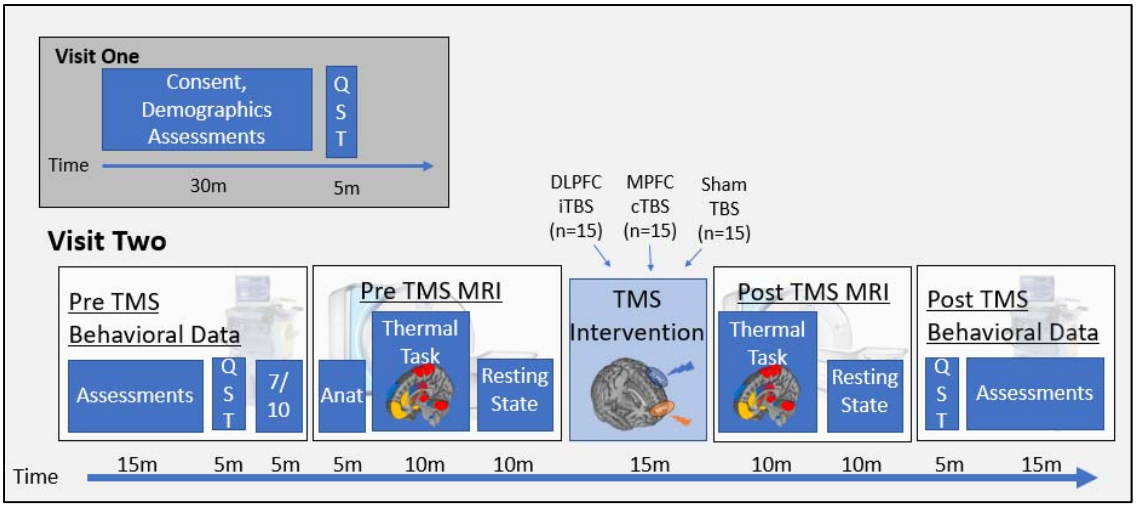

Figure 3.2 Experimental Overview. Visit One consist of consent, demographic questionnaires and an introduction to basic experimental procedures. Visit two begins with a series of assessments, and then Quantitative Sensory Testing (QST), individualized pain threshold determination, and an fMRI thermal pain task. Following a single rTMS treatment, participants repeat the experimental procedures in reverse order, finishing with assessments

We also collected Quantitative Sensory Testing (QST) data using a Medoc Pathway (Medoc Ltd, Israel). For each participant a 30 x 30 mm^2 Advanced Thermal Stimulator (ATS) probe ("thermode") was affixed to the right volar forearm, 8 cm from the wrist. At baseline, the thermode is held at a constant temperature of 32 °C. During the QST procedure, the device heats up slowly (0.5 degrees C/s) and each participant indicated when they first felt the temperature change (sensory threshold), when the temperature became painful (pain threshold) and when they could no longer tolerate it (tolerance threshold). This procedure was repeated 5 times with 10 seconds between each trial. In order to account for adaptation effects, the first trial is discarded. The measure of interest is the average of the remaining 4 trials for each threshold (sensory, pain and tolerance).

Visit 2: Participants return to the Center for Biomedical imaging. First, we applied 0.1% topical capsaicin to a 40x40mm area on the left volar forearm, 8 cm from the wrist. The

capsaicin is used to sensitize the skin, as an adapted model of allodynia (Petersen and Rowbotham 1999), resulting in lower pain thresholds. Capsaicin was left in place for 20 minutes and then removed. During this sensitization period, participants completed the BPI and POMS surveys, and we performed QST on the right arm, as done on the first visit. After 20 minutes we removed the capsaicin and performed a test to determine the temperature each participant would receive while in the MRI. The thermal stimulus rose quickly to a preset temperature, remained there for 10 seconds, and then returned to baseline. After the temperature returned to baseline, each participant provided a rating of the stimulus. We used a staircase method to determine the temperature that each participant rated a '7/10' ('painful'), corresponding to a high level of pain that can be tolerated without moving, as well as a '3/10' ('mild'), corresponding to the sensation of heat. The location of the thermode was marked on both wrists for accurate placement throughout the study. Participants then completed the same delay discounting task and Pain Questionnaire. All thermal testing was performed using the same 30x30mm thermode.

Magnetic Resonance Imaging: Participants were provided with earplugs and positioned supine in the scanner. MRI scanning is divided in the pre- and post-TMS session. For the pre-TMS session, we first collected a scout sequence for the purpose of positioning the bounding box of subsequent sequences. Next, we collected an $1mm^3$ isotropic anatomical image (MPRAGE, 256x256x192, TR/TE 2300/2.26ms) to be used for functional alignment and normalization to Montreal Neurological Image (MNI, "standard") space. Next, we collected a multiband, multiecho BOLD-sensitive functional image sequence (2.9x2.9x2.9 mm^3, TR: 1350ms, TEs 15.4, 33.66, 51.92 ms, FA 60 degrees, Acquisition time: 10:28, GRAPPA: R=2, Multiband: Factor=3, 51 slices) while the pain task was performed. Identical parameters we also used for a

resting state scan, in which participants were instructed to relax, looking at a fixation cross ('eyes open'), remain awake and try not to think of anything in particular. We also collected a short (5 volumes) identical functional image sequence with reverse phase encoding (posterior to anterior) and a field map image (2.8x2.8x2.8 mm^3, 56 slices, TR 586 ms, TEs 4.92, 7.38) to correction for distortions due to inhomogeneity in the B0 magnetic field. For the post TMS session we repeated the above sequences, excluding the anatomical, immediately after TMS stimulation.

fMRI Thermal Pain Task: For the thermal task within the scanner the 3x3 thermode was passed through a wave guide and then placed on the participant's left wrist, in the location previously marked during 7/10 testing. The thermode remained at a baseline of 32 degrees throughout the procedure with the exception of 3 participants (2 Sham, 1 MPFC), for which we used a 28 degree baseline determined during their 7/10 testing. Prior to starting the thermal task, the instructions were displayed on the screen and participants rated their current levels of Pain intensity and Pain Unpleasantness using a 5-button response pad placed in their right hand. E-Prime (Psychology Software Tools, Inc., Pittsburgh, USA; https://pstnet.com/products/e-prime/) was used to display the visual stimuli and to trigger the Medoc device. The order of painful and mild stimuli were pre-randomized, with the same order being used for pre and post TMS scanning. Thermal stimuli rose quickly, remained at the participants temperature for 10 seconds before returning to the baseline. Thermal stimuli occurred in a 15.5 second window. The rating screen was intentionally delayed in order to capture early and late stages of pain processing (Becerra, Breiter et al. 1999, Wager, Rilling et al. 2004, Price, Craggs et al. 2007, Eippert, Bingel et al. 2009, Upadhyay, Pendse et al. 2010). Hand images asking participants to rate pain intensity, pain unpleasantness and urge to use a pain reliever then appeared on the

screen for a total of 13.5 seconds (4.5 seconds for each rating, choices: None, Mild, Moderate, Intense, Severe). The ratings were followed by a pre-randomized delay (range 4 – 7 seconds), prior to the next thermal stimulus. We delivered 9 stimuli at the painful intensity and 8 at mild. Following the conclusion of the thermal task sequence, we removed the thermode from the participants wrist and collected the reverse phase encode image prior to starting the resting state scan.

Transcranial Magnetic Stimulation: After completing the first half of the MRI procedures, we walked participants to the TMS room, which is located in the same building. Subjects were randomly assigned to receive one of three stimulation types: intermittent theta burst (iTBS) to the left DLPFC, continuous theta burst (cTBS) to the left MPFC or a Sham stimulation. The stimulation location was determined on the basis of the EEG 10-20 system, with the DLPFC location corresponding to F3, and MPFC corresponding to Fp1, using an updated version (clinicalresearcher.org/eeg) of the Beam Method (Beam, Borckardt et al. 2009). Sham stimulation was performed in an 'active' manner, in that electrodes were placed underneath the coil, mimicking the discomfort associated with TMS. The MagVenture Sham system delivers electrical pulses that are derived from the stimulation pattern so that the sensation matches the TMS pulse sounds. Sham type assignment either iTBS-like or cTBS-like was randomly assigned (cTBS-like N=5, iTBS-like N=10). TMS stimulation was delivered at 110% of each participant's resting motor threshold. Stimulator intensity was ramped up quickly during the first few seconds of stimulation from a starting point of 20% of machine output in order to improve tolerability.

Following TMS stimulation, participants completed a questionnaire asking if they thought they received active or sham stimulation, their level of confidence and the pain associated with the TMS procedure. There was a significant main effect of stimulation type ($p<0.05$) on the level

of pain associated with rTMS. In examining post hoc tests, there was no difference between pain with cTBS and iTBS, however both were significantly more painful than sham stimulation ($p \leq 0.001$). There was no significant difference between confidence in correct and incorrect guesses (correct confidence: 6.4±2.77, incorrect 6.33±2.19), nor was guessing accuracy was not significantly different from chance, with only 66% of individuals guessing correctly ($\chi^2 = 3.46$, $p > 0.05$). Next participants completed the Pain Questionnaire and immediately returned to the scanner. We recorded the time between the end of the TMS session and the beginning of the thermal and rest tasks in the scanner. The average delay between the start of the pain task and end of the TMS session was 6.7±2.1 minutes (range 4 to 16). For resting state, the average delay was 18.6±1.6 minutes).

After the conclusion of the post-TMS scanning procedures, participants returned to the same screening room and again completed the delayed discounting task, a third session of QST and a fourth Pain Questionnaire. Vitamin E cream was offered in order to reduce any pain associated with experimental procedures.

MRI Preprocessing

Images were processed using a combination of tools, including dcm2niix (Li, Morgan et al. 2016), MRTrix (Tournier, Calamante et al. 2012), and AFNI (Cox 1996). First, we converted all images from the scanner DICOM format to NIfTi using dcm2niix. We also generated a skull stripped anatomical image and the deformations required for warping the data into standard MNI space using the anatomical image using AFNI's @SSWarper. We then reduced image noise using tools from MRTrix (Veraart, Novikov et al. 2016). Next, we used AFNI's automated processing pipeline (*afni_proc.py*) on the pre- and post-TMS sessions independently. The first

three volumes of each data set were discarded to ensure that images had reached steady-state and large spikes in the data were removed (*3dDespike*). Next, the data was corrected for temporal offsets in slice timing (*3dTshift*) and motion during fMRI acquisition (*3dvolreg*). The singleband reference image produced by the multiband sequence was used as a as a registration target and data from the first echo was used to calculate all transforms. Deformations to correct for inhomogeneity in the B0 field were calculated from the original and reverse phase encode singleband reference images. The functional images were then aligned to the skull stripped structural image using 6 degrees of freedom and a local Pearson correlation cost function (Saad, Glen et al. 2009). In order to reduce blurring, all transformations (motion estimates, distortion correction, anatomical alignment and MNI transformation) were combined and applied in a single step to each echo.

These separate echoes were then combined using version 3.2.2 of the ME-ICA *tedana* algorithm (Kundu, Inati et al. 2012, Kundu, Voon et al. 2017). Briefly, this algorithm determines the T2* of each voxel and then performs a weighted combination of the echoes into a single 'optimally combined' timeseries. Principal component analysis (PCA) and independent component analyses (ICA) are then performed on this data set. The independent components are then fit to each echo to determine if they show signal properties that depend on the echo time (BOLD-like) or not (non BOLD-like). Components that are identified as non BOLD-like are regressed from the data, as well as a spatially varying global signal (Power, Plitt et al. 2018), consistent with prior usage (Bethlehem, Lombardo et al. 2017, Lin, Cocchi et al. 2018, Marusak, Peters et al. 2018). The final output from this processing is two unsmoothed, MNI-space data sets, corresponding to pre and post TMS conditions.

fMRI Preprocessing: These datasets were then combined in a regression model. First, the data is smoothed using a 6 mm FWHM gaussian filter. Next, we scaled the data such that each voxel had a mean intensity over time of 100. This is performed such that parameter can be interpreted as percent signal change (Chen, Taylor et al. 2017). Regression was performed using 3dREMLfit in order to correct for autocorrelations in the timeseries, producing more accurate statistics (Olszowy, Aston et al. 2018, Chen, Polimeni et al. 2019). Models for pain events followed convention in the literature (Becerra, Breiter et al. 1999, Wager, Rilling et al. 2004, Price, Craggs et al. 2007, Eippert, Bingel et al. 2009, Upadhyay, Pendse et al. 2010), using a biphasic response model in order to account for temporal variability in the pain response, and distinguish between early and late phase responses. For this task, the early phase was modeled using the SPMG1 double gamma function, with a duration of 10 seconds, starting at stimulus onset. The model for late phase was identical but delayed by 12.5 seconds from stimulus onset. A boxcar with a duration of 13.5 seconds was convolved with the SPMG1 double gamma to model the rating period. Regressors of no interest include the motion parameters, their derivatives and the first five principle components from a subject-specific lateral ventricle mask. The primary contrasts of interest are pre vs post-TMS early pain and pre vs post-TMS late pain.

Group Analyses: We used AFNI's multivariate modeling approach, 3dMVM (Chen, Adleman et al. 2014). The contrast of painful vs mild stimulus from each subject were entered into a full model with within-subjects variables of timepoint (pre, post TMS) and phase (early, late). Treatment group was modeled as a between-subjects variable. Thresholds for Pre-TMS were set at a voxelwise $p<0.001$, with cluster significance corrected for multiple comparisons using Family Wise Error (FWE) at $p_{FWE}<0.05$. Thresholds for within group and between group comparisons were set at $p <0.005$, cluster $p_{FWE}<0.05$. For all analyses, we used Monte Carlo simulations

(*3dClustSim*) based on AFNI's ACF smoothness estimates (*3dFWHMx*) derived from task residuals (Cox, Chen et al. 2017) in order to reduce false positives (Eklund, Nichols et al. 2016).

Behavioral Analyses

Statistical analyses were performed in SPSS. (IBM). The effectiveness of randomization was assessed with a series of one-way ANOVAs examining each measure between groups. For the pre vs post TMS comparison the dependent measures are: Pain Intensity and Pain Unpleasantness ratings provided in the scanner (MRI Pain Ratings) as well as QST Pain and Tolerance measures. Paired t-tests were used to determine if there was a within-group difference on each measure, with correction for performing comparisons by group (critical $p = 0.016$) A Stimulation Type by Pain Measure ANOVA was used to determine if there were differences between groups. Significance levels for all other tests were set at $p<0.05$, two sided.

Results

There were no significant differences between the different stimulation types (all $p>0.05$) on demographic measures, thermal stimuli, resting motor threshold or delay between TMS session and post TMS scan (Table 3.1).

Table 3.1 Demographic Measures

	All Subjects n=45	**DLPFC n=15**	**MPFC n=15**	**SHAM n=15**
Age (years)	28.9±9.2	29.5±10.5	3`0.7±10.2	26.4±6.5
Education (years)	17.8±2.6	17.6±1.7	18.7±3.8	17.3±1.7
BDI	2.3±3.7	3.1±5.1	1.9±3.4	1.8±2.3
STAI State	24.2±5	24.2±3.8	24.6±6.8	23.9±4.2
STAI Trait	27.8±7.4	27.6±7.5	27.5±8.7	28.2±6.3
POMS: Mood Disturbance	14.9±12.3	11.4±8.4	13.9±10.7	19.3±16
Painful Temp (°C)	40.7±4.1	40.1±3.9	40±4	41.8±4.5
Mild Temp (°C)	36.9±3.6	36.6±3.4	36±3.5	38.1±3.9
Resting Motor Threshold	49.8±8.5	52.1±7.9	49.9±9.9	47.4±7.4
Delay after TMS (minutes)	6.7±2.1	6.9±3.1	6±1.1	7.1±1.6

Pre-TMS Behavioral Data

MRI Intensity Measures: On average, participants rated the intensity of the painful heat stimulus as a 3.73±0.73 on a 5-point scale. Unpleasantness was rated 3.33±0.83 on the same scale. Urge to use a pain reliever was rated at an average of 1.4±0.8. There were no significant differences between the assigned stimulation type groups on the pre-TMS measures of intensity, unpleasantness or urge to use pain reliever (Figure 3.3).

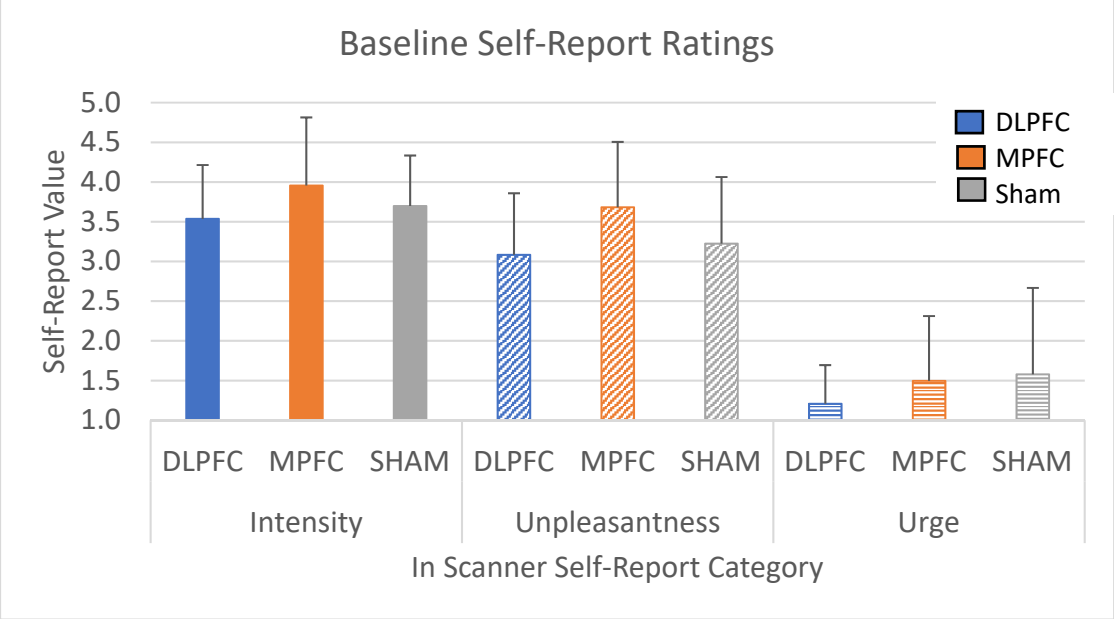

Figure 3.3 Baseline self-report ratings provided in the scanner in response to pain. There were no differences between individuals assigned to different stimulation groups on the self-reported measures of pain intensity, pain unpleasantness or the urge to use a pain reliever. Error bars show standard deviation.

QST Thresholds: On average, participants had a sensory threshold of 37.8±2.7 °C, pain threshold of 45.1±1.8 °C and a tolerance threshold of 47.3±1.7 °C (Figure 3.4) on their right wrist. There were no significant differences between groups on QST Sensory or Tolerance thresholds, however for QST Pain Thresholds, there was a main effect of stimulation type ($F_{2,42}$ = 3.621, p=0.035). Post hoc tests found that this was driven by a difference between the MPFC and Sham groups (Bonferroni p=0.037).

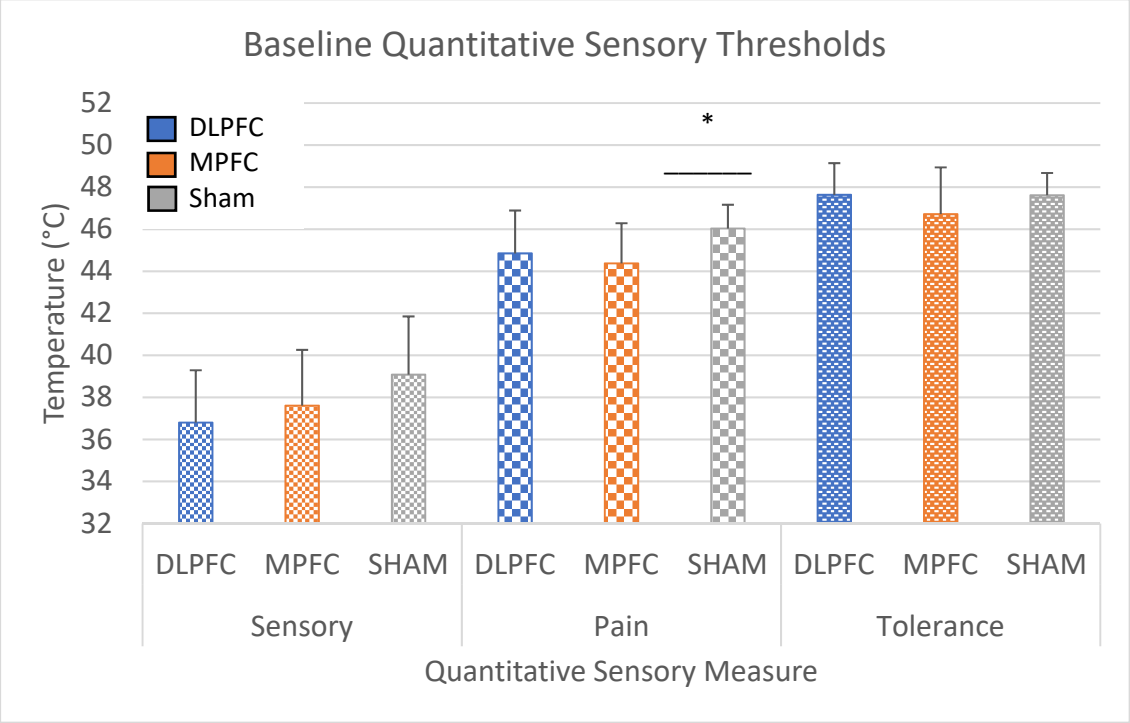

Figure 3.4 Pre-TMS Quantitative Sensory Testing Measures. Pain and Tolerance Quantitative Sensory Thresholds prior to any intervention. Error bars show standard deviation. Groups were very similar, however, there was a main effect of stimulation type for Pain Thresholds. Post hoc tests showed this was driven by MPFC vs Sham Stimulation (p=0.037).

Pre-TMS General Linear Model Results

Early Phase: Overall subjects showed positive activation in during the early phase of pain in the bilateral insula, right thalamus, anterior cingulate, dorsolateral prefrontal cortex, right caudate, right putamen, right postcentral gyrus, anterior cingulate, SMA, cerebellum (vermis, areas VI, VIII) and areas of the brainstem corresponding to the PAG and RVM (Figure 3.5A). Decreases in activity were found in the left and right superior parietal lobule, middle occipital, precuneus, right super frontal gyrus ($p<0.001$ two-sided, all clusters $p_{FWE}<<0.01$, voxels $q_{FDR}<0.005$).

Late Phase: During the late phase of pain, we found positive activation in the bilateral anterior insula, thalamus, rostral portions of the anterior cingulate cortex and medial prefrontal cortex, the right visual cortex, the vermis of the cerebellum, and the midbrain, including the PAG (Figure 3.5B) Decreases in activity were found in the left primary visual cortex, superior and middle occipital gyrus ($p<0.001$ two-sided, all clusters $p_{FWE}<0.05$, $q_{FDR}<0.05$).

There were no significant differences between the groups prior to TMS in either the early or late phase of the brain response to pain.

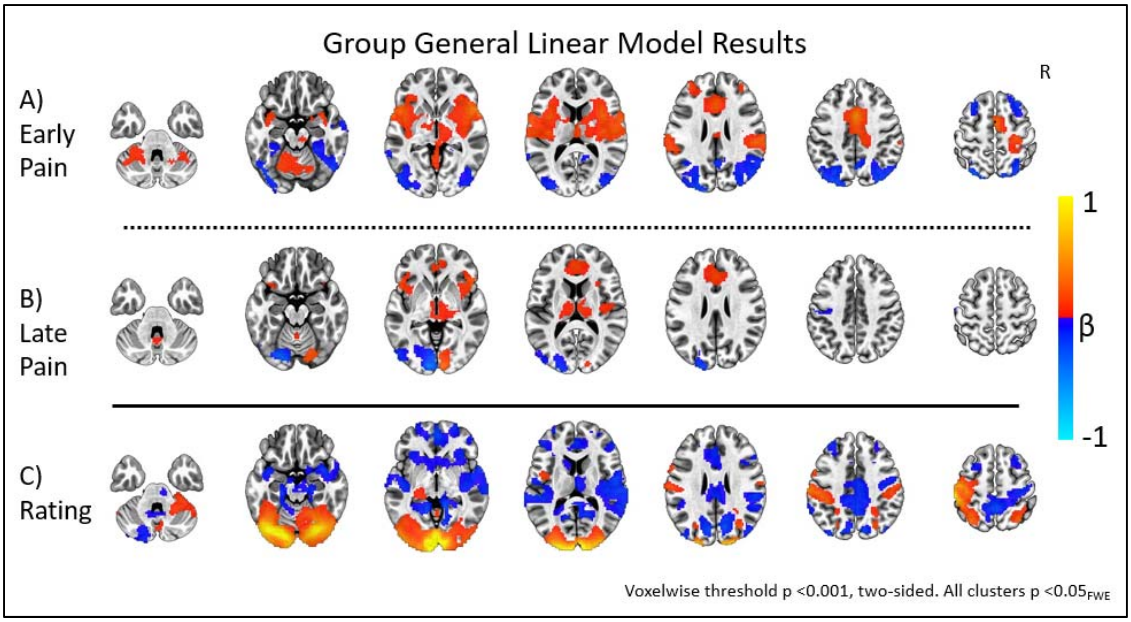

Figure 3.5 Pre-TMS General Linear Model results. Statistical Maps showing the Early **(A)** and Late **(B)** phase brain responses to the Painful vs Mild Stimulus. Positive activation in response to painful stimuli are visible in the cerebellum, brainstem, including PAG, the insula, ACC, thalamus and somatosensory cortex. In the late phase of pain processing activation is elevated in the perigenual anterior cingulate, the thalamus, the anterior insula and secondary somatosensory cortex. Bottom panel **(C)**, shows the brain activity associated with the rating period, in which participants viewed a rating screen and responded with their left hand. Activation is visible in the visual cortex, the lateral geniculate nucleus, as well as the left motor cortex and right cerebellum. Right side of the brain is on right.

Rating Block: Prior to TMS, participants had robust activation in response to the rating task. In order to aid in interpretability of the findings, the p-value threshold was raised to a voxelwise level of $p<0.00001$ (corresponding to a $q_{FDR}<<0.01$), and only clusters made up of more than 100 voxels ($p_{FWE}<<0.01$) were considered (Figure 3.5C). Participants had significant clusters of positive activation in the visual cortex, left motor cortex, right cerebellum, left lateral geniculate nucleus, left insula, right superior parietal lobule. Participants had significant negative activation in the left and right: superior temporal gyrus, secondary somatosensory cortex, cuneus, precuneus, posterior cingulate cortex, lingual gyrus, right: precentral gyrus, thalamus and left: cerebellum and anterior cingulate cortex.

There were no significant differences between the groups prior to TMS in the brain response to the rating task. Coordinates of peak T values and clusters sizes can be found in Table 3.2.

Pre vs Post TMS Behavioral Data

MRI Pain Measures: After DLPFC and Sham there was not a significant change in pain intensity (DLPFC p=0.41, Sham p=0.29) or unpleasantness (p=0.09, 0.28). After MPFC stimulation both pain intensity ($p <0.005$) and unpleasantness (p=0.0052) were significantly decreased (Figure 3.6).

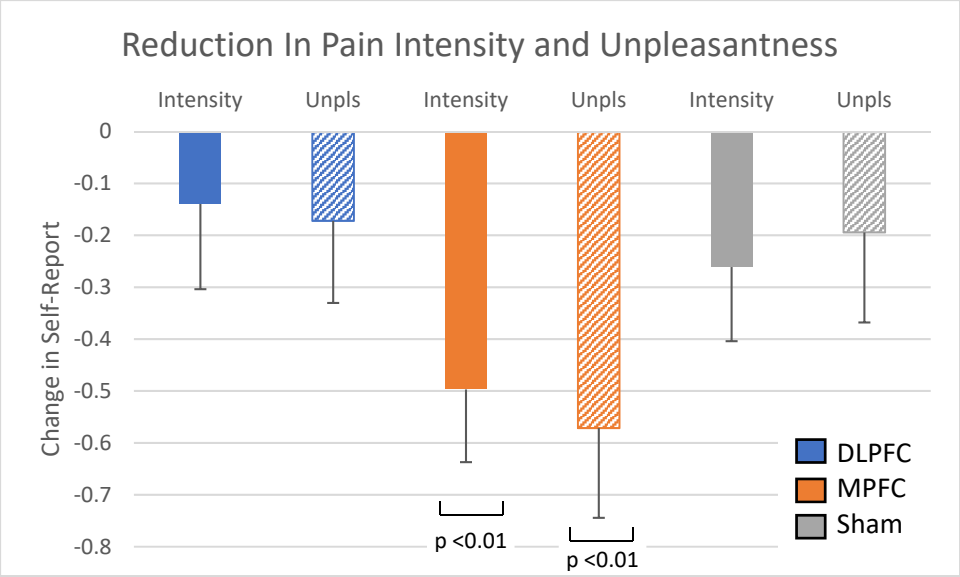

Figure 3.6 Reduction in In-Scanner Pain Responses following TMS. Neither DLPFC nor Sham stimulation led to significant changes in Self-Reported Pain intensity or Unpleasantness. MPFC stimulation led

QST Pain Measures: After DLPFC and Sham stimulation there was not a significant change in QST Pain Thresholds (DLPFC p=0.31, Sham p=0.96) or Tolerance Thresholds (p=0.73, 0.96). After MPFC stimulation QST Pain Thresholds (p=0.013) but not Tolerance Thresholds (p=0.051) and significantly elevated.

Collectively there was no main effect of stimulation type when all pain related factors were considered in a single model ($F_{8,80}$ p=0.244).

Correlations with rTMS Pain: We examined a collection of correlations in order to examine the effects of the painfulness of the treatment procedure on the change in pain measures. The relationship between the painfulness of the TMS procedure and the 1) change in Pain Intensity, 2) the change in QST Pain and 3) tolerance thresholds was non-significant. There was a significant relationship between the painfulness of the TMS procedure and change in Pain Unpleasantness (R^2= 0.1574, p=0.007). This effect appears to be driven by the sham painfulness ratings. When all zero ratings (n = 6) were removed from the analyses there is no longer a significant correlation (R^2= 0.099, p >0.05).

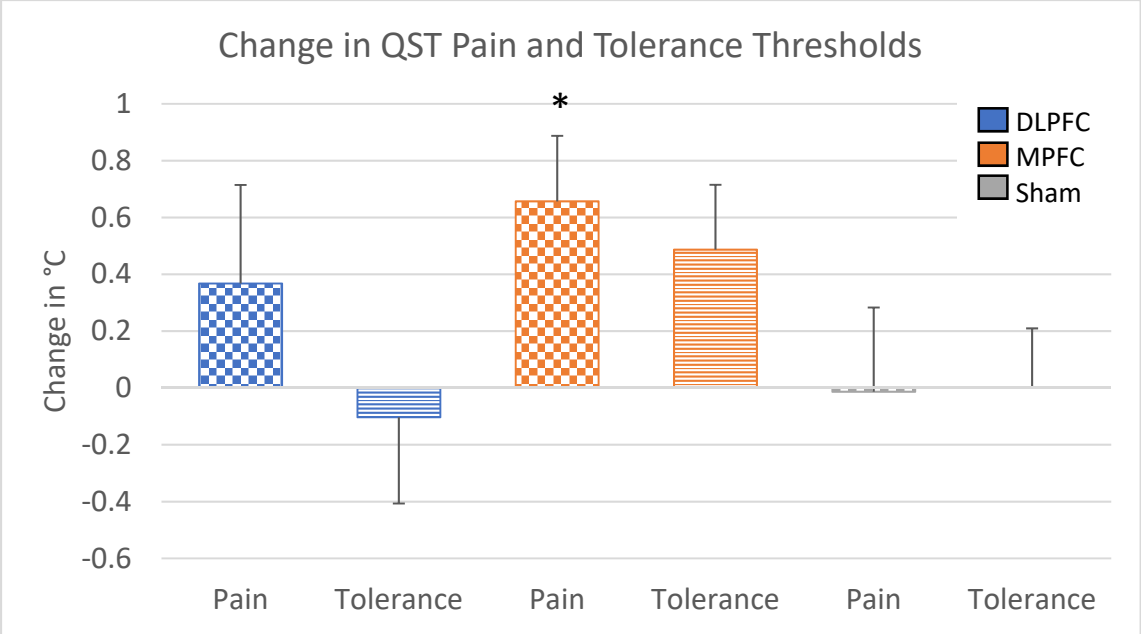

Figure 3.7 Elevation in Pain and Tolerance Thresholds After TMS. Neither DLPFC or Sham stimulation led to significant changes in QST Pain or Tolerance thresholds. MPFC stimulation resulted in a significant increase in Pain, but not Tolerance thresholds ($p = 0.013$). Error bars show S.E.M. *Indicates significant when corrected for 3 (stimulation types) comparisons.

Within Group: Pre vs Post TMS fMRI Results

Early Phase Figure 3.8**, left:** After DLFPC stimulation, there were no significant differences in the brain response to pain. After MPFC stimulation, there was significantly increased activity in two clusters. The first includes the left pre and post central gyrus. The second cluster includes the right cerebellum (Areas V, VI, VIII). After Sham stimulation, there were decreased pain vs mild response in the right cerebellum (area VI) (voxel threshold $p < 0.005$, clusters $p_{FWE} < 0.05$).

Late Phase Figure 3.8**, right:** After DLPFC stimulation, there was decreased activity in one cluster, in the ventromedial prefrontal cortex. After MPFC stimulation there was increased activity in two clusters. The first cluster included the left pre and post central gyrus as well as the superior and inferior parietal lobule. The second cluster included the right post central gyrus and superior parietal lobule. There was decreased activity in on cluster, in the perigenual portion of the anterior cingulate cortex. After sham stimulation, there was decreased activity in one cluster, in the perigenual portion of the anterior cingulate cortex (voxel threshold $p<0.005$, clusters $p_{FWE}<0.05$).

MPFC Covariates: In order to investigate the how the brain and behavior were linked in MPFC stimulation we examined the relationship between the Pre vs Post Painful vs Mild contrast with self report measures. In line with our hypothesis that MPFC stimulation would lead to larger effects on unpleasantness, we examined the relationship between the late, cognitive evaluative phase of pain processing and changes in unpleasantness. We found three significant clusters showing areas that had decreased responses as self-reported unpleasantness decreased (Figure 3.9). These were 1) the SMA, 2) the left insula and secondary somatosensory cortex, and 3) the brainstem, including the PAG, and cerebellum.

Rating Block: There were no significant differences between activation during the rating period (voxel threshold p<.005, all clusters p_{FWE}>0.05) when examined for each stimulation type.

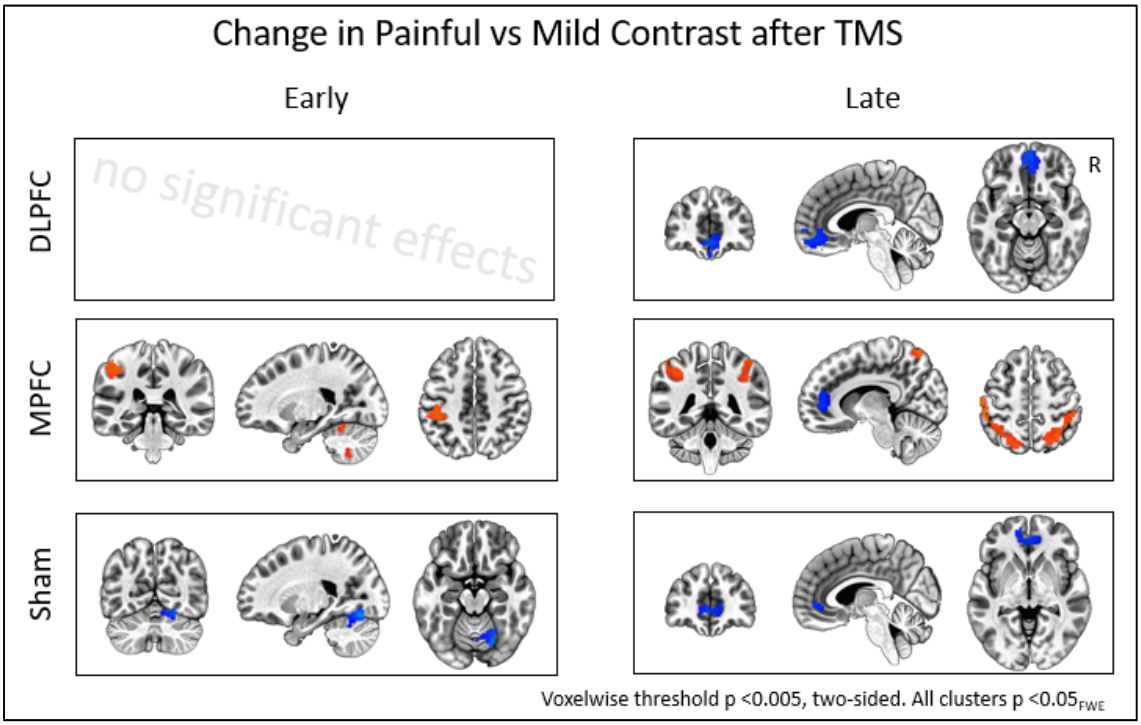

Figure 3.8 Pre vs Post Transcranial Magnetic Stimulation Painful vs Mild Brain Responses. **Left, Early Phase:** After DLPFC stimulation, there were no significant differences in the brain response to pain. After MPFC stimulation there was increased activity in sensory and motor areas in the cortex and cerebellum. After sham stimulation there was a reduction in activity in the right cerebellum. **Right, Late Phase:** After DLPFC stimulation there was a reduction in the brain response in the medial orbitofrontal cortex. After MPFC stimulation, there was bilateral increases in activity in sensory and motor cortex, as well as decreases in anterior cingulate activity. After sham stimulation there were decreases in anterior cingulate cortex activity.

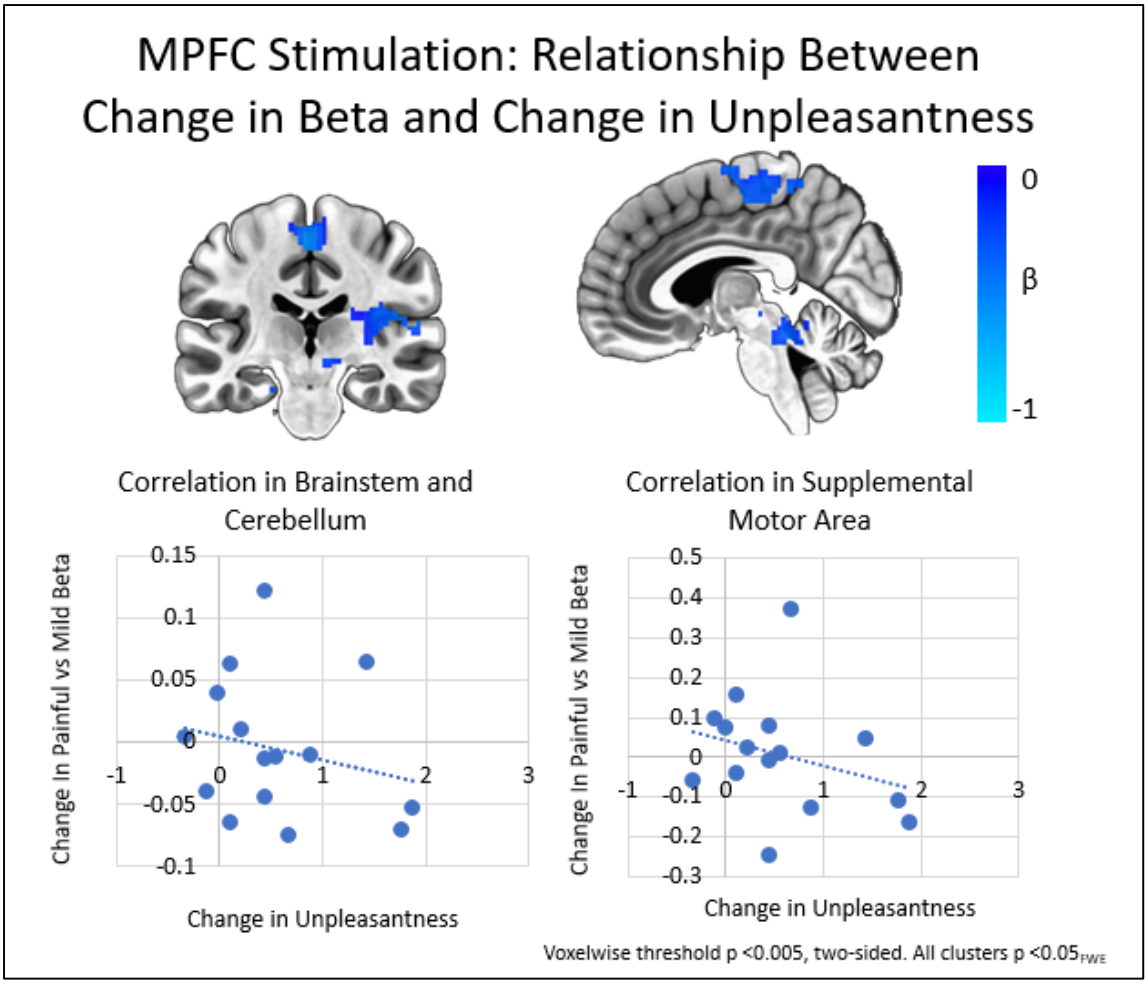

Figure 3.9 Relationship Between Brain and Behavior. Here we examine how the brain response to Painful vs Mild stimulation was related to the changes in self-reported unpleasantness. Multiple areas showed decreased responses as the participants rated the stimuli as less unpleasant, including the SMA, the right insula, secondary somatosensory cortex. brainstem and cerebellum.

Between Group: Pre vs Post TMS fMRI Results

Early Phase, Figure 3.10, top: There were no significant differences between DLPFC stimulation pre vs Post and Sham. MPFC stimulation showed a significant greater effect, relative to sham stimulation, in two clusters. The first was in right cerebellum (VI, vermis) and the other included the left pre and post central gyrus. There were no areas in which Sham stimulation showed a larger effect relative to other stimulation types.

Late Phase Figure 3.10, bottom: There were no significant differences between DLPFC stimulation pre vs Post and Sham. MPFC stimulation showed a significant greater effect, relative to sham stimulation, in one cluster during the late phase, which included the left pre and post central gyrus. There were no areas in which Sham stimulation showed a larger effect relative to other stimulation types.

Rating Block: There was also not a main effect of stimulation type or timepoint, nor was there an interaction between timepoint and stimulation type.

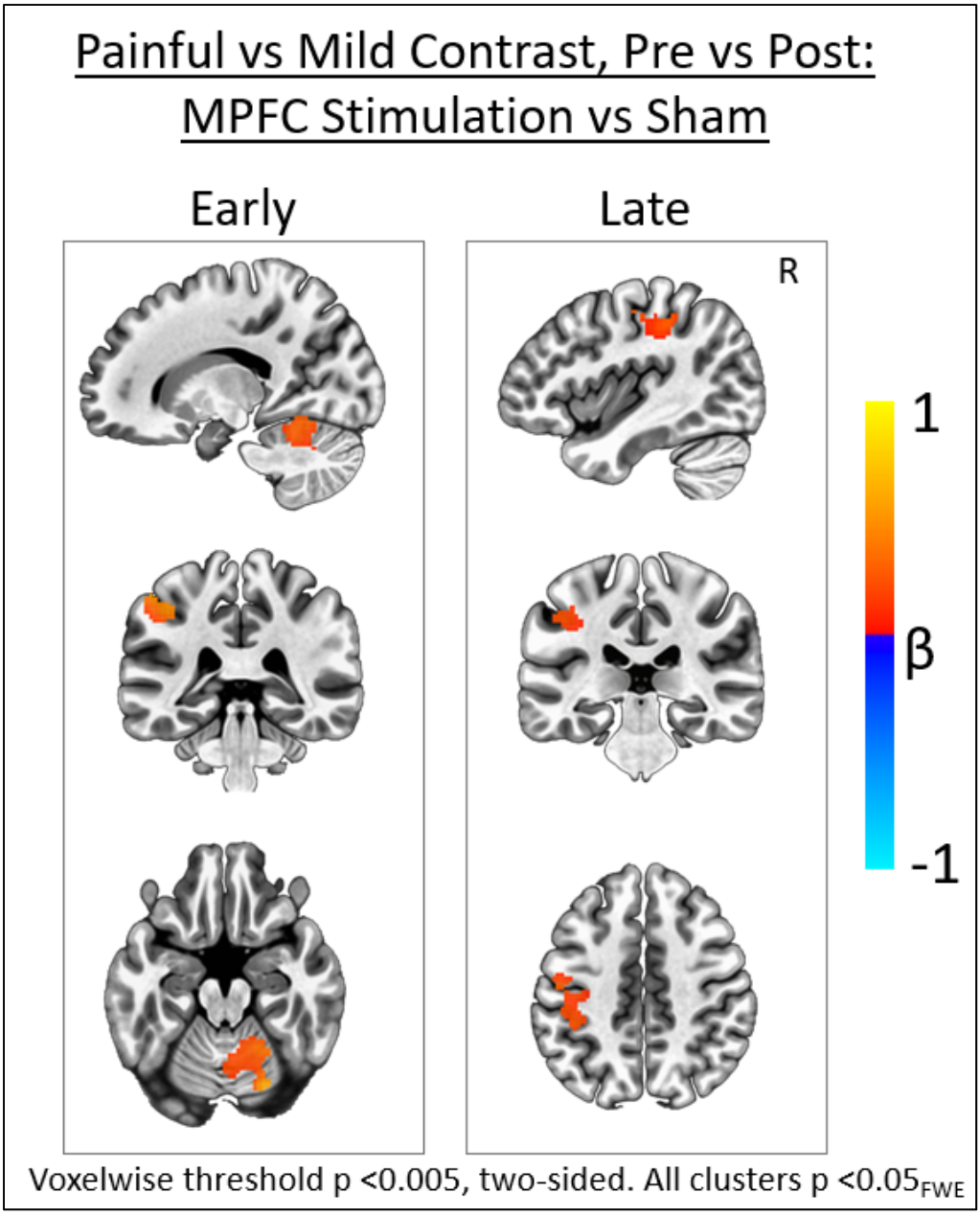

Figure 3.10 Pre vs Post Stimulation, MPFC stimulation relative to Sham. **Left** In the early phase of pain processing, we found larger increases in activity in the right cerebellum after MPFC stimulation, relative to Sham. **Right:** In the late phase of pain processing we found increases in activity in the left pre and post central gyrus after MPFC stimulation, relative to sham.

Table 3.2 Coordinates from GLM analyses

	Size	Peak T Score		
		x	y	z
Pre TMS				
Painful vs Mild:				
Early Phase				
	9916	-62.5	-3.2	5.5
	3334	0	-5.8	43
	3196	-17.5	79.2	55.5
	2593	25	76.8	55.5
	585	30	51.8	-52
	364	-2.5	61.8	38
	283	-27.5	-25.8	58
	254	-5	29.2	-49.5
	228	32.5	-53.2	28
	175	27.5	-28.2	58
	164	70	41.8	3
	142	25	44.2	-9.5
	104	-37.5	-48.2	33
	92	-15	74.2	-59.5
	89	-65	1.8	-14.5
	72	-55	-5.8	43
Late Phase				
	1820	10	94.2	-2
	1246	0	-28.2	30.5
	1237	-2.5	16.8	13
	590	-10	94.2	0.5
	570	-47.5	-20.8	-4.5
	400	50	-18.2	-4.5
	265	-2.5	44.2	-24.5
	177	57.5	14.2	53
	41	-15	39.2	33
Rating Block				
	7522	7.5	96.8	-2
	2096	-62.5	-0.8	5.5
	1721	42.5	21.8	68

	1326	0	39.2	50.5
	819	70	34.2	18
	565	-2.5	76.8	35.5
	382	-5	41.8	8
	311	12.5	46.8	-2
	301	5	79.2	35.5
	227	-35	26.8	70.5
	160	10	54.2	-62
	135	0	-28.2	28
	124	-2.5	14.2	10.5
	121	-32.5	61.8	60.5
	102	22.5	26.8	-4.5
Pre Vs Post MPFC TMS				
Painful vs Mild Early Phase				
	436	32.5	24.2	73
	204	-20	51.8	-19.5
Late Phase				
	938	55	31.8	58
	746	-17.5	69.2	68
	443	0	-58.2	20.5
Pre Vs Post DLPFC TMS				
Painful vs Mild Early Phase				
	-	-	-	-
Late Phase				
	443	0	-60.8	-4.5
Pre Vs Post Sham				
Painful vs Mild Early Phase				
	188	-20	71.8	-14.5
Late Phase				
	271	-2.5	-48.2	-2

Pre Vs Post, MPFC > Sham				
Painful vs Mild				
Early Phase				
	444	-20	74.2	-14.5
	268	32.5	24.2	73
Late Phase				
	237	50	16.8	45.5

Discussion

Summary

This study examined whether MPFC or DLPFC Theta Burst stimulation could reduce behavioral and brain responses to acute pain in thermal controls relative to a sham stimulation. We found that MPFC stimulation but not DLPFC or sham, was effective in reducing self-reported pain, elevated pain thresholds and was associated with increases in sensory and motor processing during the pain stimulus. All interventions, including sham, led to changes in the brain response to pain, but only MPFC stimulation led to significant increases in the brain response to pain over and above sham. We find support for our hypothesis that MPFC stimulation would alter behavioral pain, but did not find decreases in the brain response in standard pain areas.

Baseline Pain Processing

Dividing the pain task into early and late phase allows a closer investigation of pain processing (Becerra, Breiter et al. 1999, Wager, Rilling et al. 2004, Price, Craggs et al. 2007, Eippert, Bingel et al. 2009, Upadhyay, Pendse et al. 2010). Typically the early phase of pain related brain activation is associated with the identifying where and what the stimulus is, while the latter phase is associated with cognitive evaluation, including encoding details such as intensity (Taylor and Fragopanagos 2005, Kong, White et al. 2006, Price, Craggs et al. 2007, Moulton, Pendse et al. 2012). We find results that are consistent with the early phase response being associated with the input and sensory aspects of the pain signal. This is visible in the lateralized activation of both the thalamus and somatosensory cortex, which is no longer

present in later phases, consistent with previous work (Upadhyay, Pendse et al. 2010, Moulton, Pendse et al. 2012). During the late phase of the pain response activation is broadly reduced. Activation is more anterior along the midline, in the rostral anterior cingulate and medial prefrontal cortex, possibly indicating with more evaluative processes, including negative emotional processing (Kragel, Kano et al. 2018). In addition, activity remains elevated in the posterior insula and secondary somatosensory cortices, likely reflecting continued evaluation of the intensity of the stimulus, possibly in preparation for rating (Chen, Ha et al. 2002, Moulton, Pendse et al. 2012, Geuter, Boll et al. 2017)

DLFPC Stimulation

Previous work offers empirical support for DLFPC stimulation as a tool to reduce pain, though this has typically been evaluated using 10Hz protocols, in contrast to iTBS. There is evidence that excitatory stimulation can increase anterior cingulate blood flow (Teneback, Nahas et al. 1999) or metabolism (Baeken, De Raedt et al. 2014) in individuals with depression. In healthy controls there is evidence that 10Hz stimulation can increase dopamine release in the anterior cingulate (Cho and Strafella 2009) and that single pulses modulate the BOLD response (Dowdle, Brown et al. 2018). Despite these previous findings, we failed to find an effect in healthy controls in the anterior cingulate, or other pain processing regions during a pain task following intermittent theta burst stimulation. Though iTBS appears to be equivalent when used as a depression treatment (Blumberger, Vila-Rodriguez et al. 2018), those effects are seen only after multiple sessions. Using the motor cortex as a target, early reports suggested reliable facilitation in response to iTBS (Huang, Edwards et al. 2005), however more contemporary findings are less consistent. In recent studies, fewer than half of subjects showed the expected

facilitation following iTBS (Hamada, Murase et al. 2013, Lopez-Alonso, Cheeran et al. 2014), and that daily iTBS unexpectedly reduced the effectiveness of motor training (Lappchen, Ringer et al. 2015). Though we failed to find an effect after a single session of iTBS in healthy controls there is still support for DLPFC stimulation, particularly in clinical populations. Specifically, a single session of 10Hz stimulation resulted in patients using less morphine, as measured by patient-controlled analgesia (PCA) pump and needing less morphine long-term (Borckardt, Weinstein et al. 2006). These analgesic effects are now under intense study, with over 30 clinical trials using rTMS in order to reduce acute or chronic pain in various clinical populations (Galhardoni, Correia et al. 2015).

MPFC Stimulation

Our second intervention strategy, targeting the medial prefrontal cortex with continuous theta burst stimulation for pain, is a novel approach. The medial prefrontal cortex is emerging as a novel target for the treatment of substance abuse disorders but has not yet been explored as a treatment target for pain. There is now substantial literature linking it to pain processing. The medial prefrontal cortex also has anatomical connectivity to the PAG, similar to the DLPFC (Hadjipavlou, Dunckley et al. 2006, Kucyi, Salomons et al. 2013). When pain relief is mediated by mind wandering, this engages the default mode network, specifically including the medial prefrontal cortex node (Kucyi, Salomons et al. 2013) and in the absence of specific instructions the self-reported pain response is reduced as activity in the medial prefrontal cortex increases (Woo, Roy et al. 2015). Stronger functional connectivity between the ventral striatum and the medial prefrontal cortex was predictive of a transition to chronic back pain (Baliki, Petre et al.

2012) and the larger connectivity of this network is disrupted in patients with chronic pain (Baliki, Mansour et al. 2014).

In the healthy controls that received MPFC stimulation, we found 12.5% and 16% reductions in self-reported pain intensity and unpleasantness during scanning. For QST there was a 0.7 °C increase in pain thresholds, relative to sham stimulation. Though this was just a single intervention, and QST measures occurred later in study procedures, the effect size is roughly half that of NSAIDs (Sycha, Gustorff et al. 2003).

We were unable to confirm the neuroimaging aspects of our hypotheses. Despite reductions in behavioral scores of pain, we did not see changes in typical pain processing regions. Instead, we found increases in activity across motor and sensory areas, as well as the parietal cortex. These activations are dissimilar to many other types of pain relief, which find decreases in insula and ACC activity after opioid administration (Wise, Rogers et al. 2002, Bingel, Wanigasekera et al. 2011), placebo (Wager, Rilling et al. 2004), or imaginative distraction (Schulz, Stankewitz et al. 2019). Given that we targeted a key node of the default more network (DMN), the medial prefrontal cortex, it is also possible that increases in other nodes, such as the inferior parietal cortex could reflect successful modulation of that circuit. These activations occurred during both phases of pain, and are spatially similar to activity associated with shifting attention to non-imaginative distractions during pain (Schulz, Stankewitz et al. 2019). In this study, no instructions were provided in regards to the heat pain task. Further work will be required to uncover the relationship between MPFC stimulation and pain relief, with one promising possibility including combining MPFC stimulation with specific pain relief instructions (such as distraction or revaluation).

Though we did not evaluate a pharmacological intervention in this study, the correlation between decreases in PAG activity during the late phase of pain and lower unpleasantness ratings suggest a possible role of endogenous opioids, as has been seen following DLPFC stimulation. Prior work found midbrain decreases following active TMS, that could be blocked using naloxone, an opioid antagonist (Taylor, Borckardt et al. 2013). The same relationship was found in the posterior insula and secondary somatosensory cortices, further linking these regions with the encoding of particular aspects of the stimulus (Chen, Ha et al. 2002, Moulton, Pendse et al. 2012, Geuter, Boll et al. 2017).

Sham Stimulation

The limited differences following sham stimulation in both early and late phases of pain processing suggest that the task reliably produced pain responses at both timepoints. Notably, the average effect on pain and tolerance thresholds was nearly zero, which may reflect a lack of adaptation.

Limitations

No strategies were provided to participants, nor did our questionnaires capture how individuals dealt with pain with the fMRI environment. Future studies may benefit from capturing or directly manipulating pain strategies, in order to evaluate the effectiveness of stimulation. A clear limitation of the present study is that sham was significantly less painful relative to both active types of stimulation, possibly confounding results. Despite this difference, participants were unable to guess which stimulation type was received, nor were there any difference in correct or incorrect guesses.

Conclusion

Stimulating the medial prefrontal cortex with continuous theta burst stimulation may be an effective new target for pain relief. In the current work, MPFC stimulation reduced both behavioral and brain responses to an acute thermal painful stimulus in healthy controls, with greater activity in sensory in motor areas relative to sham stimulation. This work has important implications for developing brain stimulation for pain, however, there is at least one more important consideration. Many individuals who have chronic pain and use prescriptions opioids over a long period of time may have altered responses to the pain experience. Modifying pain in healthy controls is only the initial step, and the next chapter explores how patients with chronic pain and opioid use may differ from a population of controls.

Chapter 4 Examining Differences Between Healthy Controls and Chronic Pain Patients

Introduction

In 2014, there were 245 million prescriptions for opiates written in the United States (Volkow and McLellan 2016). Unfortunately, the widespread availability of these powerful analgesic drugs has led to a public health crisis and increases in mortality and morbidity associated with chronic prescribing. In 2015, 33,000 individuals had fatal overdoses caused by licit and illicit opioids (Rudd, Seth et al. 2016). Despite recent success in reducing the overall number of prescriptions, prescribing rates have remained high (Guy, Zhang et al. 2017). The increased availability of opiates places many individuals at risk of conversion to opiate use disorder (Volkow, Benveniste et al. 2018), and with chronic use individuals are susceptible to a paradoxical increased sensitivity to pain known as opioid-induced hyperalgesia (Lee, Silverman et al. 2011, Nusrat, Yadav et al. 2012).

Decades of preclinical work has elucidated several mechanisms by which opiate usage can lead to states of hyperalgesia (Simonnet and Rivat 2003, Ossipov, Lai et al. 2005, Angst and Clark 2006, Roeckel, Le Coz et al. 2016). One commonly uncovered mechanism operating at the peripheral and spinal levels is NMDA-dependent, long-term potentiation (LTP) (Drdla, Gassner et al. 2009, Zhou, Chen et al. 2010) at nociceptive afferents. These findings have been translated to clinical practice, with meta-analyses supporting the effectiveness of ketamine, an NMDA antagonist, in reducing post-surgical pain (Wu, Huang et al. 2015). Preclinical work has also uncovered alterations at the supraspinal level, with evidence for faciliatory, pronociceptive activity within the rostral ventromedial medulla and periaqueductal gray (Vanderah, Suenaga et

al. 2001, Rivat, Vera-Portocarrero et al. 2009), as well as increases in protein kinase activity across the cortex (Sanna, Ghelardini et al. 2014). In humans, the supraspinal mechanisms through which chronic prescription opiate usage alters brain reactivity to pain are not well understood, though neuroimaging is uncovering the regions involve in pain processing. Functional magnetic resonance imaging (fMRI) studies of acute pain in healthy individuals demonstrate that there is a reliable network of brain regions (the "Pain Matrix") which are engaged by an acutely painful stimulus (Apkarian, Bushnell et al. 2005, Wager, Atlas et al. 2013, Cauda, Costa et al. 2014, Tanasescu, Cottam et al. 2016). These brain regions include: (1) the anterior cingulate cortex (ACC) and insula, which are primary nodes in the "Salience Network" (Seeley, Menon et al. 2007); (2) the somatosensory cortex and thalamus, which are primary sensory processing areas and their subcortical afferent; (3) as well as prefrontal regions and brainstem nuclei (Melzack 2001, Petrovic, Petersson et al. 2004). Positron emission tomography (PET) studies demonstrate that several of these areas have high endogenous opiate receptor levels, including the ACC (Vogt, Watanabe et al. 1995), insula (Baumgartner, Buchholz et al. 2006), and thalamus. Additionally, acute experimental pain evoked with the application of a thermal stimulus leads to an increase in opiate receptor binding specifically in the ACC and insula among healthy individuals (Sprenger, Valet et al. 2006). A recent meta-analysis demonstrated that the brain response to acute pain in chronic pain patients is similar to healthy controls (Tanasescu, Cottam et al. 2016). Notably, however, none of these studies examined how opiate usage affects the pain response, with many studies excluding patients who use opiates. Given that the brain regions involved in processing acute pain contain high levels of opiate receptors, it is possible that chronic opiate use in individuals with chronic pain may lead to homeostatic dysregulation in this system.

The purpose of this pilot study was to evaluate the pattern and amplitude of neural activity associated with acute pain in a sample of chronic pancreatitis patients that have been using opiates daily for 6 or more months. Chronic pancreatitis is a particularly intransigent condition associated with visceral pain. Similar to other chronic pain conditions, pain originates from a specific location, but over time the etiology of this pain spreads. In part, this may be due to alterations in central processing, as chronic pancreatitis is associated with changes in brain structure in pain processing regions (Dimcevski, Schipper et al. 2006, Dimcevski, Sami et al. 2007, Dimcevski, Staahl et al. 2007, Bouwense, Ahmed Ali et al. 2013), mimics neuropathies (Dimcevski, Sami et al. 2007, Staahl, Dimcevski et al. 2007, Drewes, Krarup et al. 2008), and surgical intervention is not guaranteed to resolve pain symptoms (Rosch, Daniel et al. 2002, Cahen, Gouma et al. 2007). Given these difficulties, opiates are frequently prescribed to treat chronic pancreatitis (Goulden 2013, Kleeff, Whitcomb et al. 2017). Little is known, however, about the effects of chronic opiate use on the processing (behavioral and neurobiological) of acute pain in this population. Given the goal of developing a non-opiate based therapeutic for these patient populations, evaluating the neural response to pain in these patients, compared to a control population, is a necessary and important step.

Materials and Methods

Participants and Questionnaires

All procedures for this research were reviewed and approved by the Medical University of South Carolina's (MUSC) Institutional Review Board. Individuals with chronic non-alcoholic pancreatitis ('patients', n=14, 10 female) currently using chronic opiates (>6 months) were

recruited from the MUSC Pancreatitis Clinic. Non-opiate using control individuals ('controls', n=14, 10 female) were recruited from the local community. Following informed consent, participants completed a demographic questionnaire, the Brief Pain Inventory (BPI; (Cleeland and Ryan 1994)), and the Current Opioid Misuse Measure (COMM; (Butler, Budman et al. 2007)). The Medoc Pathway System (Medoc Ltd, Ramat Yishai, Israel) was used to identify a hot temperature (°C) that each participant rated as a 7 out of 10, corresponding to an intense pain that could be tolerated without moving. This testing was done using a slightly adapted model of hot allodynia (Petersen and Rowbotham 1999). Specifically, 0.1% capsaicin cream was applied to a 40 x 40 mm area of the skin 12 cm from the wrist on the left volar forearm. After 30 minutes the cream was removed, and 7 out of 10 testing was performed in the capsaicin sensitized region. Heat was delivered using a 30 x 30 mm ATS thermode.

Table 4.1 Opioid Misuses and Brief Pain Inventory Rating for Patients

Current Opioid Misuse Measure (COMM)	
Total	9.9±7.5
Prescribed Opiate Counts	
Oxycodone	11
Morphine	4
Hydromorphone	3
Hydrocodone	3
Fentanyl Patch	2
Methadone	4
Brief Pain Inventory (BPI)	
Medication Relief (%)	63.6±26.0
General Activity	5.2±3.1
Mood	3.9±3.0
Walking Ability	2.9±2.8
Normal Work	5.2±3.7
Relationships	3.6±3.3
Sleep	5.1±3.6
Enjoyment	4.1±3.5
Concentration	4.0±3.2
Appetite	4.4±3.9

MRI Data Acquisition

Each participant was positioned supine in a Siemens 3T TIM Trio, with their head positioned in a 12-channel head coil and secured by foam. Up to three runs of blood oxygen dependent signal (BOLD) data, reflecting functional brain activation, were acquired (TA: 13:12 36 slices, TR 2.2s, 35ms TE, FA: 90 degrees, 3x3x3mm). A high resolution T1-weighted, MPRAGE anatomical image was also collected (TE: 4.18ms, TR 1.75s, $1mm^3$ voxels).

Pain Task Design

The experimental design is illustrated in Figure 4.1. During each experimental run, the thermode was placed on the capsaicin sensitized region of the left forearm and response buttons were positioned on both hands. During the first 5 minutes and 57 seconds of each run, the temperature alternated between a baseline of 32° (19 seconds), or each participant's 7/10 temperature (14 seconds). Following each block of heat, participants performed a control task (button press) to ensure they were remaining awake, with their eyes opened. These tasks were repeated 8 times per run, after which participants self-reported overall pain intensity and pain unpleasantness. There were three sequential runs of the task. All participants completed all three runs with the exception of four patients and two controls (who completed two runs due to a delay in starting the fMRI acquisition).

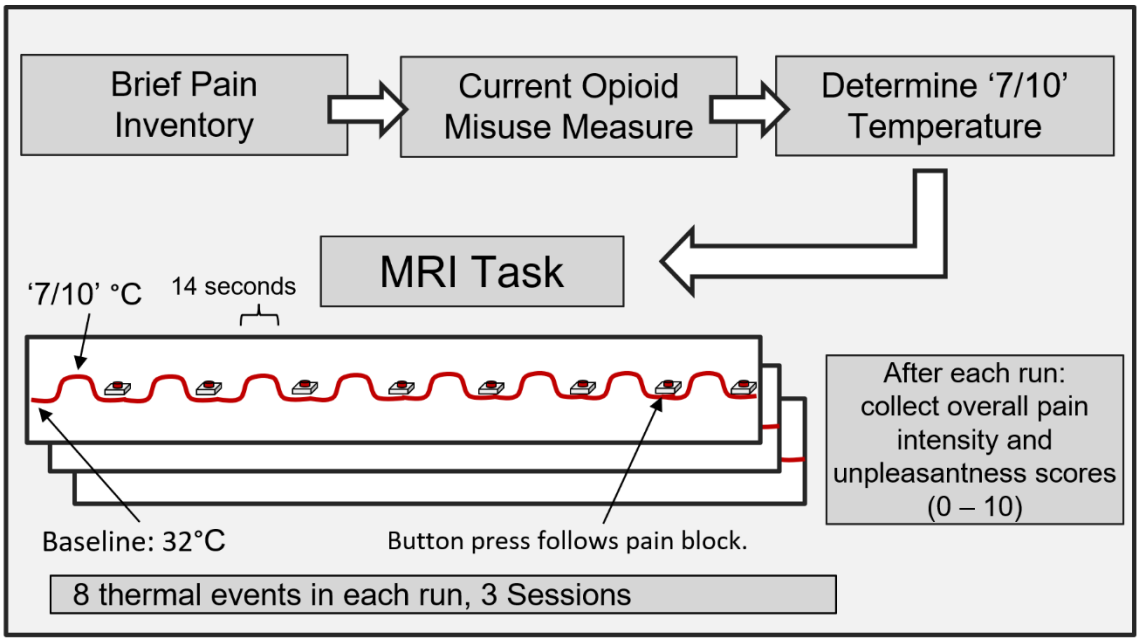

Figure 4.1 Basic Task Design. On the study visit, participants completed a series of assessments prior to the MRI task. The Thermal task consisted of up to 3 runs, with 8 thermal events in each run, wherein the temperature corresponded to each participants '7/10' pain threshold. A button pressing control task occurred after each pain block. After each run, participants provided pain ratings.

fMRI Data Processing

The data were converted from DICOM format to NIfTI using dcm2niix. SPM12 running in Matlab 2017a (Mathworks) was used for rigid-body timeseries realignment. The mean images produced by realignment were used to perform normalization directly to MNI space using the EPI template provided with SPM, which may improve registration outcomes (Calhoun, Wager et al. 2017). Images were smoothed using an 8mm FWHM gaussian kernel and exported to the CONN functional connectivity toolbox version 17.f (Whitfield-Gabrieli and Nieto-Castanon 2012). Consistent with prior work (Kucyi, Moayedi et al. 2014, Zeidan, Emerson et al. 2015, Flodin, Martinsen et al. 2016), we extracted the first 5 principle components (PCs) from eroded white matter and cerebral spinal fluid regions. These PCs were then regressed from the smoothed timeseries (i.e. CompCor; (Behzadi, Restom et al. 2007). Simultaneously high pass filtering (cutoff 100s) and the 6 realignment parameters with first order derivatives were also regressed.

The data were then used in subject-level general linear models to determine BOLD signal change due to (1) pain and (2) button pressing. Contrast maps produced by regression against a double gamma hemodynamic response function convolved with the task design were carried forward to a two-sample t-test. To examine other effects on pain response in the patient group, we performed a within-group model that included age, 7/10 temperature, current pain, morphine mg equivalents (MME) and total COMM score as covariates. Due to the slice prescription in some subjects, much of the occipital cortex and cerebellum were excluded. Statistical analyses followed typical fMRI methods, applying an initial threshold to limit the analyses to a subset of all the voxels (voxel threshold) and then determining significance by examining contiguous collections ('clusters') of voxels that survive that threshold. Within-group

analyses used a voxel threshold of $p<0.001$, reporting clusters that were $p_{FWE}<0.05$. Between-group analyses used a voxel threshold of $p<0.01$, reporting clusters >150 voxels. The covariate analyses used a voxel threshold of $p<0.005$, reporting clusters that were $p_{FWE}<0.05$.

Self-Report Data

The effect of the thermal stimulation on self-reported pain sensation (intensity, unpleasantness) was evaluated using a general linear model with group (patients vs. controls) as the between-subjects factor and fMRI run (1-3) and self-report type ("Intensity" or "Unpleasantness") as within-subject factors (SPSS software Ver. 25, IBM).

Results

Demographics and Pain Characteristics

No group differences in gender were revealed (10 women and 4 men in both groups), however patients were older than controls (patients 48.8±8.2 years vs. controls 37.1±13.2 years, $p<0.05$). In comparison to the control group, the patient group had significantly higher scores on the BPI, including subscales for current (patients 3.4±3.4 vs. controls 0.2±0.8) and average pain (patients 5.1±2.3 vs. controls 0.8±1.2; p's<.005; Supplementary Table S1 for details). No group differences in the individual-tailored pain threshold were revealed (patients 42.4±3.0 vs. controls 41.9±4.3). The patient group was prescribed 133.5±94.8mg morphine equivalents at the time of the study, with medication taken an average of 13.7±20.2 hours prior to scanning (self-reported range: 1-77 hours).

fMRI GLM Results

Within-Group fMRI Responses

Pain Task. During pain processing, the control group and the patient group had elevated BOLD responses in several established nodes of the "Pain Matrix" including the left motor/sensory cortex, insula, prefrontal areas, and the ACC (voxel threshold $p<0.001$, cluster $p_{FWE}<0.05$; see Figure 2). Detailed coordinates are included in Table 4.2.

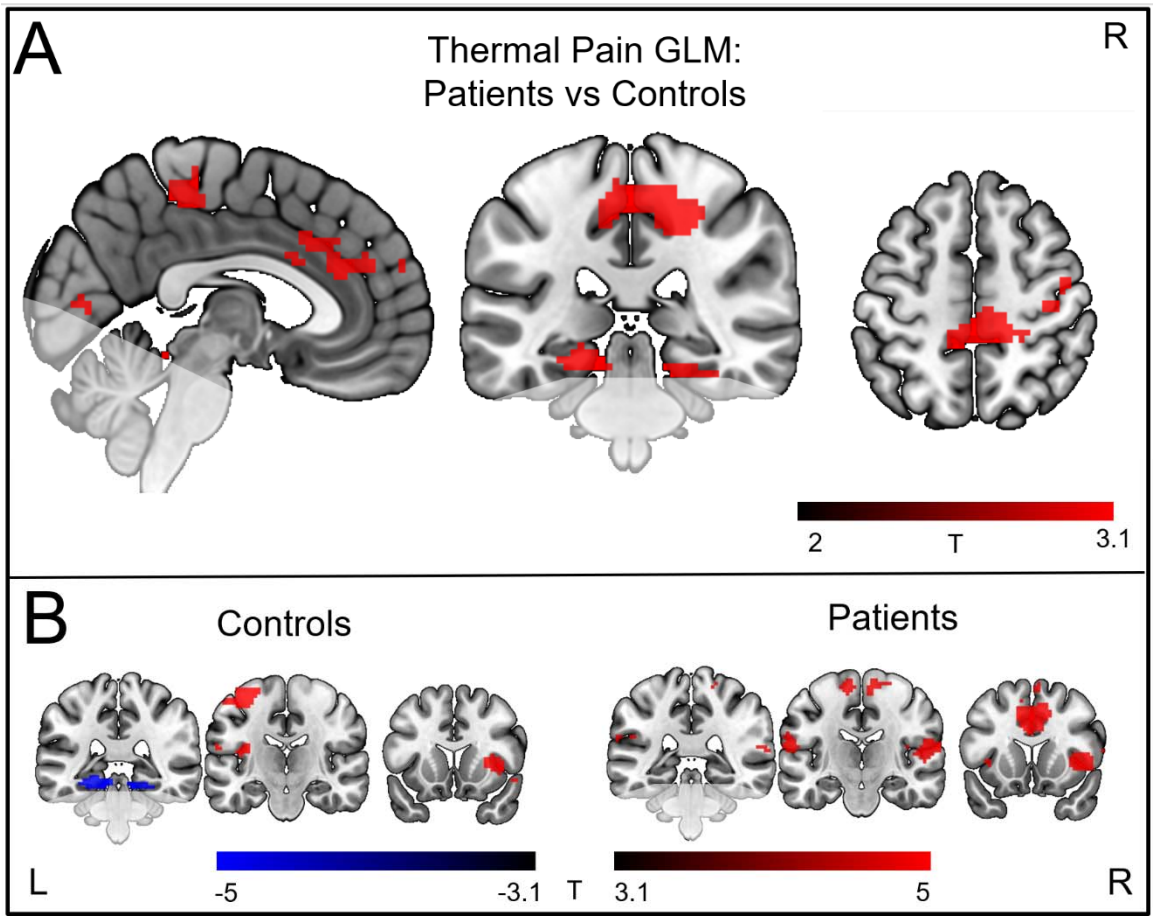

Figure 4.2 Results from GLM Analyses. **Panel A shows regions in which the pain response in the patient group was elevated relative to the control group (voxel wise p<0.01). Patients showed elevated brain responses to pain in the anterior cingulate cortex and sensory and motor regions. Panels B and C show the brain responses within group. Both groups show conventional pain activation during the thermal pain task, with activation in sensory regions, the ACC and bilateral insula.**

Motor Control Task. During the button pressing task, both controls and patients had elevated activity in the primary motor and sensory cortices, anterior cingulate cortex, bilateral thalamus and insula (p_{FWE}<0.05, Figure 4.3)

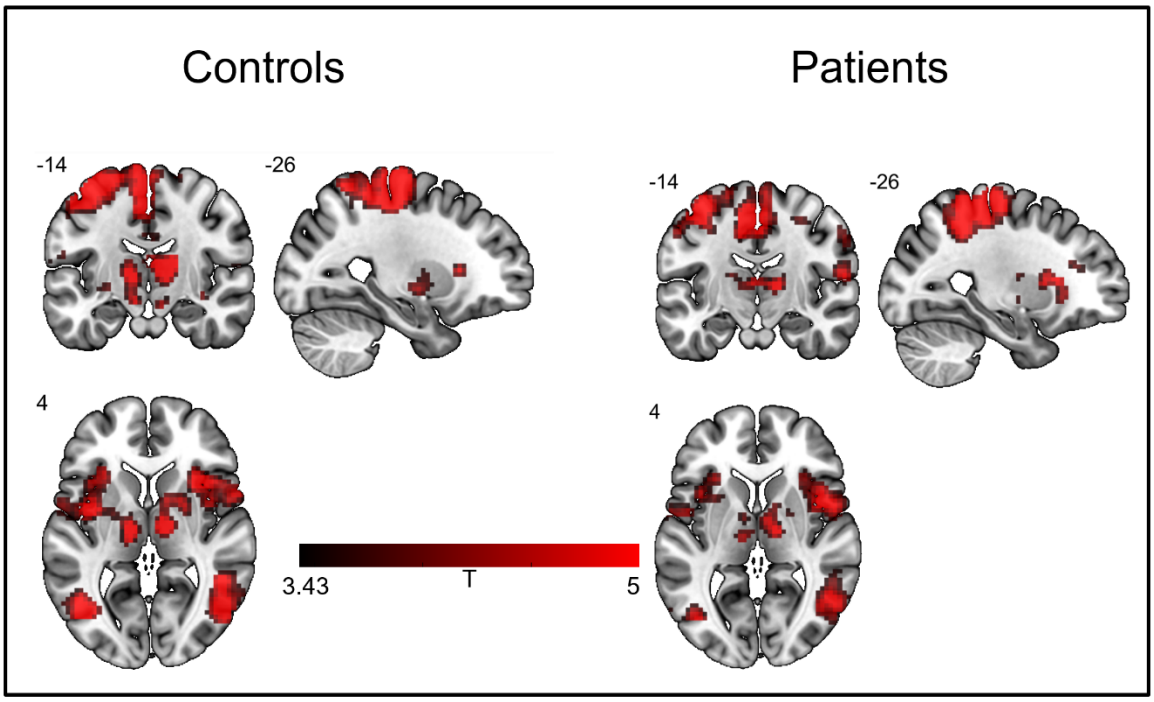

Figure 4.3 GLM results from Button Pressing Task. The button pressing portion of the task was included in order to confirm that patients were continuing to remain awake and attentive. Both controls and patients had the expected responses in the contralateral motor cortex, as well as extended activation throughout the brain. There were no differences between groups.

Table 4.2 Results from analyses of response to pain with General Linear Model Within group activation reported at a cluster forming threshold of p<0.001, minimum cluster size 60. Between group comparison reported at a cluster forming threshold of p<0.01, minimum cluster size 150. Covariates were examined with a cluster forming threshold of p<0.005, minimum cluster size 150. Any p-value reported by SPM12 as zero has been changed to a value of <0.001.

Supplemental Table 2. Results from GLM analyses of BOLD response to pain

Cluster Statistics			Cluster Locations	Peak Location (MNI)		
$p_{FWE\text{-}corr}$	Number of voxels	$p_{uncorrected}$		x	y	z
Controls Only						
<0.001	241	<0.001	R. Ant. Insula, R Central and Frontal Operculum	36	8	8
				21	26	5
				51	-1	5
<0.001	284	<0.001	L. Precentral, L. Postcentral	-36	-28	68
				-36	-22	59
				-51	-22	59
0.047	75	0.007	L. Post. Insula, L. Central Operculum,	-36	-16	14
				-51	-28	26
				-66	-22	20
0.004	137	0.001	B. SMA	-3	-7	59
				15	-1	62
				-6	8	44
Patients Only						
<0.001	625	<0.001	R. Ant. Insula, R. Central Operculum, R. Frontal Operculum	51	2	-1
				42	5	2
				57	-16	14
<0.001	1110	<0.001	B. SMA, B. Mdl. Cingulate,	6	2	68
				0	14	41
				-6	8	44
0.059	70	0.009	R. Precentral	42	-4	56
				48	-7	47

0.011	111	0.002	L. Central Operculum, L Ant. Insula, L. Precentral	-51	2	-1
				-54	-4	11
				-36	11	-1
0.034	83	0.005	L. Postcentral, L Planum Temporale, L SMG	-60	-19	14
				-63	-31	20
				-63	-19	23
Patients Greater than Controls						
0.002	550	<0.001	B. Precentral, R. Postcentral	24	-34	44
				18	-19	44
				48	-7	44
0.024	354	0.001	L. Lingual, B. Calcarine, L. Cerebellum	-24	-43	-10
				-6	-49	-10
				9	-64	11
0.252	184	0.011	R. Sup. Frontal, B. Anterior Cingulate, B. Middle Cingulate	3	35	23
				12	56	23
				30	29	23
Patients: Morphine Equivalence Covariate						
0.031	166	0.001	B. Anterior Cingulate, B. Mdl. Cingulate	6	17	35
				-9	14	32
<0.001	1133	<0.001	R. Angular, R. Mdl. Occipital, R. Mdl/Sup. Temporal, R. SMG	33	-19	8
				57	-49	14
				42	-73	11
<0.001	391	<0.001	B. Sup. Frontal, L. Mdl. Frontal, B. SMA	15	8	68
				-15	-4	68
				-12	-19	71
0.009	216	<0.001	L. Precentral, L. Central Operculum, L. Postcentral, L. Mdl. Frontal	-54	-4	17
				-48	-10	38
				-42	-7	32
0.015	194	0.001	L. Supramarginal, L. Parietal Operculum	-45	-37	23
				-57	-37	29
				-51	-37	11
0.013	199	0.001	R. Precentral, R. Postcentral, B. Precuneus	9	-28	62
				18	-22	71
				9	-19	56

Patients: BPI: Current Pain, Negative Correlation						
0.011	206	<0.001	R. Insula, R. Central Operculum	57	-10	14
				33	-1	11
				57	-19	23
R: Right, L: Left, B: Bilateral, Ant: Anterior, Post: Posterior, Mdl: Middle, Sup: Superior, SMG: Supramarginal Gyrus, SMA: Supplemental Motor Area						

Between-Group fMRI Responses

Relative to controls, patients had significantly greater activity during the thermal stimulation blocks in 3 clusters: bilateral primary somatosensory cortices (cluster $p_{FWE}<0.01$), left lingual gyrus and calcarine sulcus (cluster $p_{FWE}<0.05$), and the bilateral anterior and middle cingulate (cluster $p_{uncorr}=0.011$) (voxel threshold $p<0.01$, see Figure 2A). There were no areas in which patients showed significantly less activation compared to controls. For the button press task there were no significant differences in brain activation or reaction time (Controls 727.4±163.8ms; Patients 759.6±132.2ms ($p = 0.37$)) between groups.

Self-Reported Pain Measures during the MRI task

The average pain intensity after each fMRI run was 6.9±1.6 in the controls and 6.7±1.3 in the patients. The average pain unpleasantness was 6.7±1.7 in the controls and 5.8±2.1 in the patients (see Table 4.3for all ratings). There was no interaction between group and fMRI run, nor a main effect of group or run. There was a significant main effect of self-report type ("Intensity" and "Unpleasantness", p=0.043), as well as increasing self-report values over sessions ($p = 0.047$), however there were no group interactions.

Table 4.3 Behavioral Pain Measures

	Controls	**Patients**	**Significance**
Pain Intensity			
1st Run	6.8±1.7	6.5±1.2	n.s.
2nd Run	6.9±1.4	6.5±1.5	n.s.
3rd Run	6.8±2.0	7.5±1.6	n.s.
Pain Unpleasantness			
1st Run	6.2±2.2	5.4±2.6	n.s.
2nd Run	6.8±1.6	5.7±2.2	n.s.
3rd Run	6.8±2.0	6.4±2.5	n.s.

Relationship between Pain and Opiate Dose on the Brain Response to Acute Pain

Morphine milligram equivalents (MME) were positively correlated with the response to pain in several clusters ($p_{FWE}<0.05$). These included (1) the bilateral anterior and middle cingulate (a node in the "Pain Matrix" which was also different between controls and patients (Figure 4.2A), (2) the right supramarginal/angular gyrus, (3) bilateral superior frontal cortex, (4) left primary motor/sensory cortex, (5) left supramarginal/angular gyrus, and (6) right primary motor/sensory cortex. In order to place the results in an more interpretable context, we determined the correlation between each participants MME and the average beta from the anterior cingulate cluster. MMEs explained 32% of the variance in the beta values, which reflect the magnitude of the brain response. With the most extreme MME values (>250) removed, 47% of the variance was explained.

Current scores on the BPI were negatively correlated with the response to pain in one cluster: the right insula (also a node in the "Pain Matrix", $p_{FWE}=0.011$, Figure 4.4B). We examined the correlation between the Current Pain measure of the BPI and the average beta in the right insula cluster. The Current Pain scores on the BPI explained 53% of the variance in the brain activity in that location. With the most extreme value removed (BPI 0, Beta > 0.3) the correlation remains significant, explaining 58% of the variance. There were no significant correlations between the brain response to acute pain and age, individually calibrated pain threshold, or COMM score.

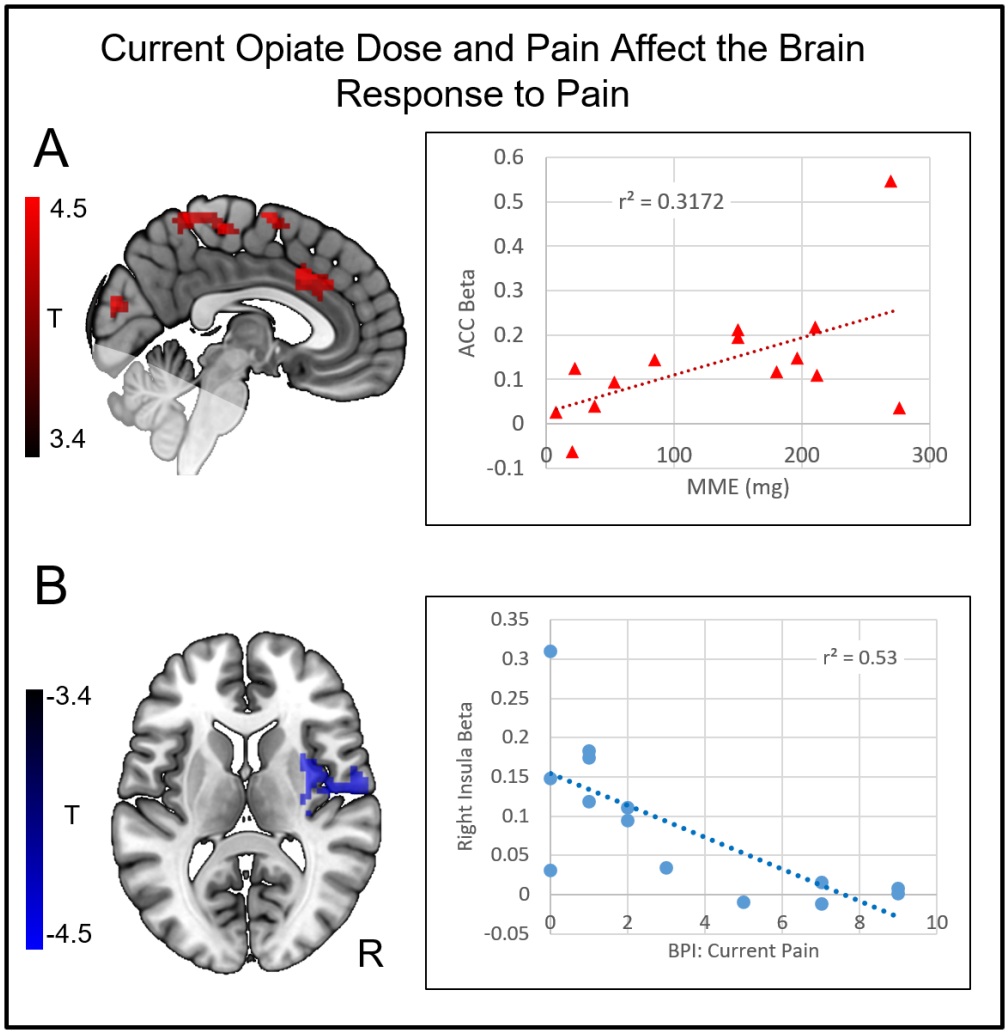

Figure 4.4 Examination of Covariates. A) Red clusters indicate areas in which the BOLD response to pain was positively correlated (pFWE<0.05) with morphine mg equivalence (MME) values (cluster forming threshold p<0.005). Color maps indicate T values. These areas include somatosensory and cingulate regions. A plot is shown indicating the relationship between the effect size (average beta from indicated cluster) and MME. MMEs explained nearly 32% of the variance in the brain response within the cingulate. The correlation remains significant following the removal of the most extreme values, with an R2 of 0.47. B) The blue cluster shows an area in the right insula in which the response to pain was negatively correlated (pFWE <0.05) with the Current Pain score, as derived from the Brief Pain Inventory. Color maps indicate T values. A plot is shown indicating the relationship between the effect size (average beta from cluster) and the Current Pain score. Current pain explained 53% of the variance in the brain response. The correlation remains significant following the removal of the first extreme value, with an R2 of 0.58. No other correlations were significant (Age, COMM score, 7/10 Temp). For the axial slice, the right side of the image corresponds to the right side of the brain. Faded areas indicate regions that were not available for analyses.

Discussion

Summary

While acute opiate usage is associated with acute pain relief, chronic opiate usage leads to a sensitized behavioral response to pain. Acute pain leads to elevated activity in a network of neural regions (e.g., the ACC, insula, and thalamus) that also have high opiate receptor concentrations. Very little is known, however, about the effects of chronic opiate usage on the brain response to acute pain. This study is the first to demonstrate that a dose of opiates that normalizes the behavioral response to acute pain in chronic pancreatitis patients is associated with an amplified neural response to acute pain. As expected, and consistent with prior reports, acute pain evoked a similar pattern of neural activity in the "Pain Matrix," with particularly high levels in the somatosensory cortex, but the amplitude of this response was elevated in opiate using patients. Furthermore, the higher levels of activity in the ACC and insula associated with acute pain were positively correlated with morphine equivalent dose. That is, the higher the prescribed dose of opiates, the larger the pain response. The findings suggest that, while these patients are taking a dose of opiates that normalizes their behavioral response to pain (e.g., they did not report feeling more intense pain than controls), there is a sensitized brain response to pain in several key neural nodes which are not only key elements of pain processing circuitry but also locations of high opiate receptor binding. The findings provide a foundation for future longitudinal investigations which may seek to investigate if this homeostatic dysregulation may, in turn, contributes to the dangerous process of behavioral tolerance and opioid dose escalation.

Increased Responses in Patients using Chronic Opiates

In the present study, both patients and controls showed typical responses to pain, with activation in somatosensory areas, the insula and the ACC. However, the patient group had amplified brain responses compared to the control group. One cluster encompassed somatosensory regions, which provide information about the location and intensity of pain (Apkarian, Bushnell et al. 2005, Lee and Tracey 2010). These areas, including the secondary somatosensory cortex (SII), also showed a positive relationship between opiate dose and pain-related activity. The SII has previously been targeted with an inhibitory form of non-invasive brain stimulation which led to reduced pain scores compare to a sham stimulation (Fregni, Potvin et al. 2011). These overlapping findings may reflect changes in sensory processing from continued use of opiates. Future longitudinal work will be needed to determine if these somatosensory responses are indicative of an increased risk of hyperalgesia development. The cluster showing elevated activity in posterior regions of the brain suggests that there may also be changes in other aspects of sensory systems, including the representations within the cerebellum or visual processing areas, but interpretation of these findings is made difficult by the limited field of view.

The third cluster differentiating patients from controls encompasses the middle and anterior cingulate cortex (ACC). Activity in these regions is associated with motivational-affective processing of pain and tracks the unpleasantness of the stimulus (Rainville, Duncan et al. 1997, Price 2000, Apkarian, Bushnell et al. 2005). The affective dimension of pain may be a key component of visceral pain. For example, an EEG study in pancreatitis patients found decreased latencies of pain-related event potentials in the ACC (Dimcevski, Sami et al. 2007), and research

in irritable bowel syndrome, found increased ACC blood perfusion in response to painful stimuli (Mayer, Berman et al. 2005).

Relationship Between Prescription, Pain and the Pain Response

The positive relationship observed between the amount of opiates prescribed and the neural response to pain highlights the potential risks associated with chronic opiate use. The cluster showing a positive correlation between dose and pain response in the ACC and middle cingulate directly overlaps with an area showing elevated activity in patients relative to controls. Although preliminary, these findings offer a functional correlate for previously found structural changes (Frokjaer, Bouwense et al. 2012). Collectively, the findings from this line of research support the idea that differences between groups in pain responses may be driven, in part, by increased opiate usage. This may reflect homeostatic dysregulation of the opiate system, such as the cingulate, as well as other areas such as the insula, thalamus and brain stem which are highly enriched with opiate receptors (Vogt, Watanabe et al. 1995, Baumgartner, Buchholz et al. 2006, Corder, Castro et al. 2018). Positive correlations were also found in motor and sensory cortices. As similar regions also differentiated the patient group from the controls, this may reflect the role that prescription opiates play in reducing the intensity of pain. Positive correlations in the supramarginal and angular gyrus may reflect alterations that opiates have on other aspects of pain processing. These regions are not typical included in the "Pain Matrix," but nevertheless show relationships with pain, such as when expectations regarding pain are violated (Zeidan, Lobanov et al. 2015, Kokonyei, Galambos et al. 2018), or participants evaluate pain intensity (Kong, White et al. 2006). Overall, the distribution of the correlations throughout the brain is likely related to the widespread presence of pain processing activity (Atlas, Lindquist

et al. 2014), and is similar in extent to changes seen in white matter structure in individuals using prescription opioids (Upadhyay, Maleki et al. 2010).

One other measure, current pain, as indexed by the Brief Pain Inventory, also showed significant correlations with pain responses. Specifically, pain on the day of scanning was negatively correlated with a single cluster in the right posterior insula. This builds upon previous reports that chronic pancreatitis patient's pain responses were shifted to a more posterior portion of the insula (Dimcevski, Sami et al. 2007), and may relate to structural changes in that area (Frokjaer, Bouwense et al. 2012).

Implications for Opioid Use Disorder

The importance of these pilot data are underscored by the overlap in neural regions involved in processing acute pain as well as those associated with processing drug-cue reactivity (Becker, Gandhi et al. 2012, Navratilova, Atcherley et al. 2015, Elman and Borsook 2016, Mitsi and Zachariou 2016). Many individuals continue to use opiates due to concerns about pain (Barth, Maria et al. 2013). Previous work supported the idea that the presence of pain was protective against the development of an addiction phenotype (Colpaert, Meert et al. 1982, Lyness, Smith et al. 1989, Colpaert, Tarayre et al. 2001, Ozaki, Narita et al. 2004), however there is growing evidence that this is not the case (Ewan and Martin 2013, Zhang, Tao et al. 2014, Hou, Cai et al. 2015) and epidemiological studies find evidence of misuse and addiction among individuals with chronic pain (Vowles, McEntee et al. 2015). This study provides possible targets for treating the pain that is associated with chronic opiate usage. Building upon prior work that stimulated secondary somatosensory areas (Fregni, Potvin et al. 2011), these findings support

non-invasive brain stimulation targeting primary somatosensory regions or regions able to modulate the anterior cingulate cortex.

Relationship with Opioid Receptor Locations

Opioid receptors are highly distributed throughout the brain (Corder, Castro et al. 2018), but the insula is particularly implicated in pain processing and is also enriched with opiate receptors (Baumgartner, Buchholz et al. 2006). To explore the relationship between the pain task used in the current study and opioid receptor binding we have compared the spatial extent of the BOLD response to pain (relative to rest) in healthy controls with previous work using positron emission tomography (PET). In Sprenger, Valet et al. (2006), [18F] DPN (a non-selective opioid ligand) was used as a tracer to measure changes in endogenous opioid binding during pain in 8 healthy controls, compared to 8 other control subjects at rest. They found that both the left and right insula showed reduced [18F] DPN ligand binding, suggesting endogenous opioid binding occurred at those locations (Figure 4.5A). These insula locations overlap with the increased BOLD response found in the current work Figure 4.5B), linking the current pain task BOLD response with locations of pain-sensitive opioidergic activity.

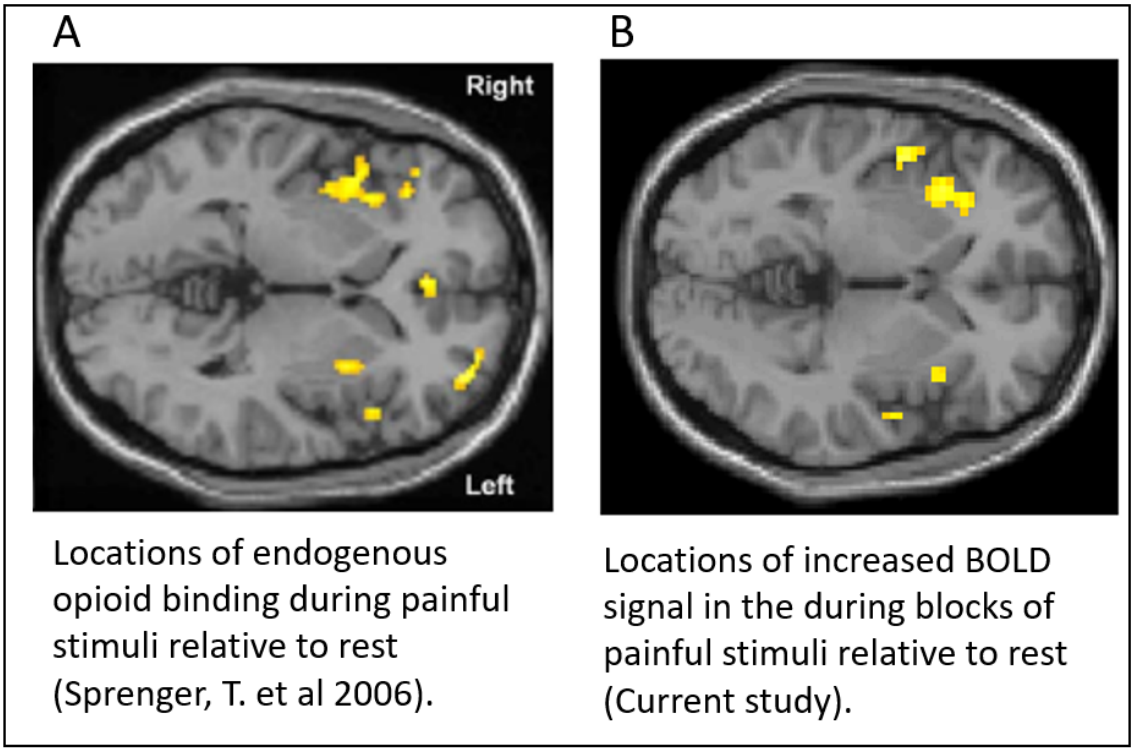

Figure 4.5 Comparison between opioid binding and BOLD response to pain. **A)** Adapted from Sprenger et al, (2006). Eight healthy controls were exposed to painful heat stimulus while opiate receptor binding was evaluated with a PET scan using [18F]DPN. These data were then compared to a separate group of healthy controls at rest. Endogenous opioid binding was found in both the left and right insula. **B)** In our study we evaluated the BOLD signal associated with pain blocks versus no pain (rest) blocks. Our findings within both the right and left insula correspond spatially to the locations of greater endogenous opiate binding found in Sprenger, T. et al 2006.

Limitations

The findings are preliminary with a relatively small sample size, and did not capture pain responses in the brainstem and cerebellum. Future work should leverage advances in fMRI sequences to determine if these pain processing and modulating regions also show altered activity in patients using chronic opiates. It will also be important to incorporate other stimulus types (e.g., cold or mechanical pain) to confirm that the findings are broadly applicable and not specific to heat-induced pain.

Conclusion

Individuals using chronic prescription opiates for pain have elevated neural responses to a thermal pain stimulus relative to healthy controls. The amount of opiates used, as measured by morphine milligram equivalents, is positively associated with larger brain responses to pain. These findings need to be explored in a larger sample and over a longer period of time to determine whether and how chronic opiate usage may increase risk for conversion to nonmedical prescription opioid use, opioid use disorder, or opioid-induced hyperalgesia.

Chapter 5 Conclusions and Future Work

This collection of studies lays a foundation for the development of a brain-based treatment for pain. Chapter 2 provides data on a paired comparison between active stimulation and a matched control demonstrating that transcranial magnetic stimulation is able to have causal effects within the brain, following targeted neural circuitry. Furthermore, in Chapter 3 we report findings from an investigation on how using a specific modulatory pattern of stimulation known as continuous theta burst can reduce the behavioral responses to an acute painful stimulus, with changes in the BOLD signal suggesting a role of motor or attentional mechanisms. These finding are then placed in an important context, as Chapter 4 reports that individuals with chronic pain and opiate usage may have elevated affective responses to pain, and that these larger responses appear driven by opioid usage.

The Importance of Causal TMS Findings

Over the many years of research using concurrent or interleaved TMS/fMRI, no studies had reported a direct comparison between active stimulation and a well-matched control stimulation condition. For our purposes, this comparison was essential to show the neural correlates of TMS stimulation at a clinically relevant target. Usage of a control condition allowed the subtraction of nonspecific effects associated with the TMS pulses, such as the noise and surprise, uncovering the BOLD response specific to active TMS. There are two main points to highlight from this work. The first is that the inactive, control stimulation resulted in a very similar spatial pattern of activation as compared to active TMS. These effects are a confound for studies seeking to report the causal effects of TMS activity as they are unrelated to the magnetic

field. As we continued to explore the effects of TMS within the brain to explore novel stimulation targets or probe circuitry, it remains essential to control for these non-specific effects.

Despite these similarities between active and control stimulation, the comparison reveals that active stimulation leads to elevated activity in regions hypothesized from classic frontostriatal connectivity models (Middleton and Strick 2000). This supports the causal, circuits-based approach to developing TMS as a treatment. While the motor cortex provides evidence that TMS is able to activate connected regions, it is necessary to extend these findings to areas that have different distributions of neuronal types, layers and connectivity. Knowledge that TMS pulses are carried by the intrinsic network of the brain allows researchers to consider treatments based on secondary connections, rather than a single specific location. This better matches the development of brain stimulation treatments to the dynamic, communicative nature of the brain (Preti, Bolton et al. 2017), rather than limiting it to static single locations.

Translating Causal Effects to Clinical Purposes

Building on the proof-of-principle work from Chapter 2, we next explored how two different cortical targets may affect pain processing. While there is prior support for using the DLPFC as a TMS target for 10Hz stimulation to reduce pain (Taylor, Borckardt et al. 2012, Taylor, Borckardt et al. 2013), no study had yet to explore theta burst stimulation at this location. Intermittent theta burst stimulation (iTBS) is thought to have similar effects compared to 10Hz stimulation (Huang, Edwards et al. 2005, Blumberger, Vila-Rodriguez et al. 2018), but with a reduced time burden. With the DLPFC target, we hypothesize decreases in the pain response in the anterior

cingulate, given data from the single pulses in Chapter 2, as well as evidence from PET following 10 Hz stimulation (Cho and Strafella 2009).

For the second target, we considered an entirely novel location for pain relief, the medial prefrontal cortex (MPFC). While this target has not yet been explored in the context of analgesia, it is a key node in the default mode network (Raichle, MacLeod et al. 2001) and is thought to play a key role in multiple evaluative processes involving reward (Dunlop, Hanlon et al. 2016), emotion (Etkin, Egner et al. 2011) and fear (Schiller and Delgado 2010). In addition to this important position in brain function in healthy controls, the MPFC is the node in the default mode network that appears to be most dysregulated by chronic pain (Baliki, Geha et al. 2008, Baliki, Mansour et al. 2014, Reddan and Wager 2018). Here we used a continuous form of theta burst stimulation (cTBS), based upon previous findings from the lab that supported reductions in TMS evoked responses in the bilateral insula (Hanlon, Dowdle et al. 2017) – key pain processing regions.

In line with current best practices (Klein, Treister et al. 2015), we also used an active sham stimulation condition that mimics the sensations of the TMS pulses. For this condition, we randomly assigned individuals to receive either 1) sham cTBS stimulation at the MPFC or 2) sham iTBS stimulation at the DLFPC.

We found that MPFC stimulation, but not DLPFC or Sham led to significant 1) reductions in self-reported pain intensity and unpleasantness, as well as 2) increases in thermal pain thresholds as measured using quantitative sensory testing. All three interventions led to changes in the brain response to pain, however only MPFC stimulation had effects larger than sham. Specifically, following MPFC stimulation participants had elevated activity in the right cerebellum and left sensory cortex, motor cortex and inferior parietal lobule. While these

changes in the brain response were inconsistent with our hypothesis, they do suggest that there are neural correlates to the behavioral changes that merit future study.

Despite prior success with DLPFC stimulation, we failed to find an effect following this stimulation condition. It is possible that iTBS effects differ from those of 10Hz in regards to pain processing. In 10 Hz stimulation, there is a clear evidence for cumulative effects of stimulation (Schulze, Feffer et al. 2018), so it is also possible that a single session of iTBS was insufficient to alter pain processing and more sessions are needed. This population is also a group of healthy controls without any known deficits in pain processing.

One final consideration is that in all cases no instructions regarding the pain experience were given. It is possible that combining the stimulation methods with a pain strategy or coping mechanism would lead to larger effects. As an example, there is evidence that DLPFC stimulation using another type of non-invasive electrical stimulation is more effective when combined with cognitive behavioral therapy (Powers, Madan et al. 2018).

Chronic Pain Populations Differ from Healthy Controls

A treatment that was only effective for healthy controls would be a partial victory. In order to extend this treatment and its findings to the population most threatened by the ongoing opioid crisis we investigate pain processing in individuals with chronic opioid use – in this case a clinical sample suffering from chronic pancreatitis. Notably, both groups responded identically to behavioral probes of painfulness, despite the presence of chronic opioid use and pain in the clinical sample. Only when the functional brain responses to pain were examined did we find that the clinical population had elevated responses to pain in sensory and affective areas.

As alluded to in the opening chapter, there remain unanswered questions regarding how continued prescription opioid usage relates to pain processing. In this study, we were able to relate important clinical variables, such as morphine equivalence values (an index of the amount of opioids prescribed), to pain processing. Specifically, in the clinical sample the brain responses to pain grew larger as the individual's dosage of opiates increased. This supports the idea that opiate usage may be having an effect on endogenous opioid signaling, which could contribute to future opioid induced hyperalgesia. In contrast to this, we found that increasing levels of pain on the day of experimental procedures was associated with decreased responses in the insula during the pain task. This suggest that there are opposing processes at work in individuals with chronic pain taking prescription opioids.

Collectively these differences between healthy controls and patients showcase the importance of evaluating pain in these samples. For this study, the patients represented a relatively homogenous sample, in that they were all suffering from chronic pancreatitis. It is necessary to extend these results into a more representative sample of individuals with chronic pain.

Evaluating Feasibility in Chronic Pain Patients

In order the evaluation whether rTMS can have an effect in a clinical population we have collected pilot data on 15 individuals with chronic pain and a history of opioid use. This clinical sample underwent a series of procedures as outlined in Chapter 3 and were randomly assigned to received either DLPFC stimulation (n=6), MPFC stimulation (n=5) or sham stimulation (n=4). The data were analyzed in an identical manner as the healthy controls presented in Chapter 3. Specifically, the data underwent preprocessing and first level modeling in AFNI to examine the

early and late responses to pain. Given the previous evidence that ongoing pain and opiate use may alter pain processing we also examined the effects of pain on the day of scanning (from Brief Pain Inventory) and prescription opioid dose, indexed via milligram morphine equivalents (MME) as was done in Chapter 4.

Results

There were no significant differences between the individuals assigned to different stimulation types (no main effect, all $p>0.05$) on demographic measures, thermal stimuli, resting motor threshold or delay between TMS session and post TMS scan (Table 5.1). Overall, participants were prescribed 145±201 Morphine Milligram Equivalents (MMEs) and on average experienced moderate daily pain with values of 4.5±2.4 on a 10-point scale, ranging from mild to severe (Brief Pain Inventory).

Table 5.1 Demographics Table for Chronic Pain Group

	All Subjects n=15	**DLPFC** n=6	**MPFC** n=5	**SHAM** n=4
Age (years)	50.1±11.3	54.2±10.7	50.6±8.4	43.3±14.6
Education (years)	14.9±3.1	14.5±3.7	15.2±3.9	15±1.2
BDI	13±12.1	11.8±11.6	12.2±11.1	15.8±16.8
STAI State	35.5±12.2	37.3±12.2	29.4±9.4	40.3±15.2
STAI Trait	37.6±14.2	35.3±12	39±16.7	39.3±17.6
POMS: Mood Disturbance	44.8±36.6	11.4±8.4	13.9±10.7	19.3±16
Painful Temp (°C)	40.6±4.3	39.6±3.9	41.6±4.3	40.9±5.6
Mild Temp (°C)	36.8±3.7	37.5±4.6	37.4±3.4	35±2.4
Resting Motor Threshold	53.1±7.6	54.3±10.7	52±2.8	52.5±7.7
Delay after TMS (minutes)	7.1±1.1	7.3±1.6	7.2±0.4	6.8±1
MME	145±201	218±252	110±207	80±82
BPI: Average	4.5±2.4	4.7±2.4	4.2±2.8	4.8±2.4
BPI: Pain at time of MRI Scan	4.9±2.9	5±2.8	5±3.4	4.5±3.3

Pre-TMS Behavioral Data

MRI Intensity Measures: On average, participants rated the intensity of the painful heat stimulus as a 3.75±0.63 on a 5-point scale. Unpleasantness was rated 3.46±0.8 on the same scale. Urge to use a pain reliever was rated at an average of 1.93±1.05 (Figure 5.1). There were no significant differences between the individuals assigned to different stimulation types on the pre-TMS measures of intensity, unpleasantness or urge to use pain reliever.

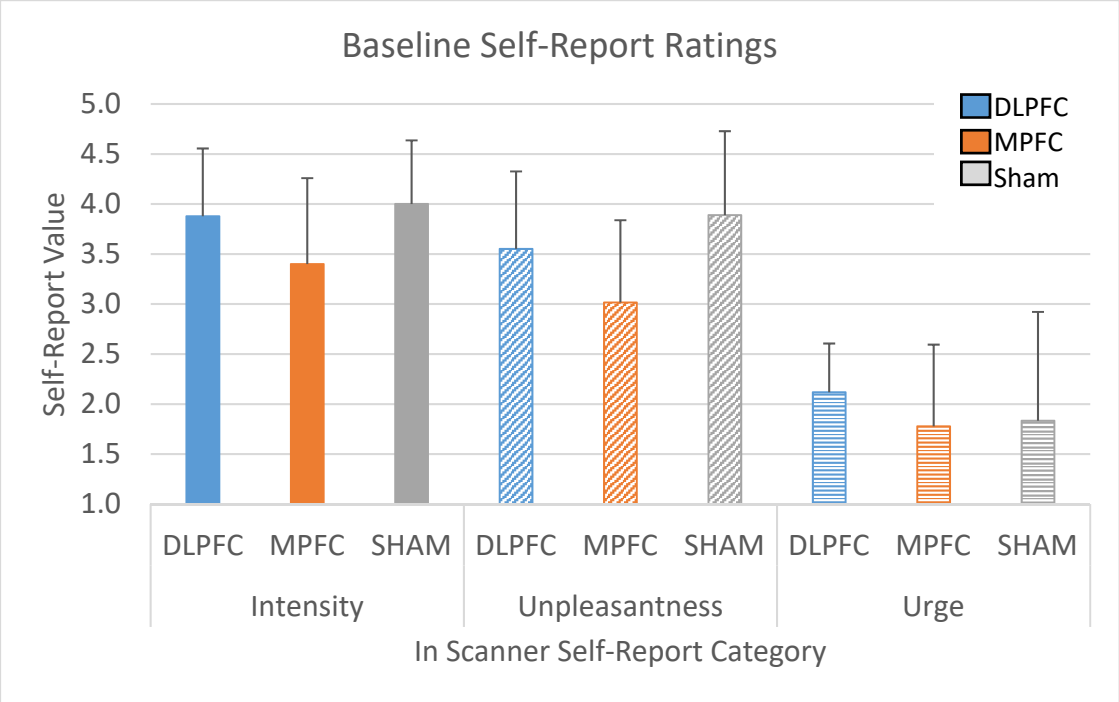

Figure 5.1 Patients Baseline Self-Report. There were no differences between individuals assigned to different stimulation groups on the self-reported measures of pain intensity, pain unpleasantness or the urge to use a pain reliever. Error bars show standard deviation.

QST Thresholds: On average, participants had a sensory threshold of 41.4±1.9 °C, pain threshold of 46.4±1.2 °C and a tolerance threshold of 48.5±1.4 °C (Figure 5.2) on their right wrist. There were no significant differences between groups assigned to different stimulation types on Sensory, Pain or Tolerance thresholds measured by QST.

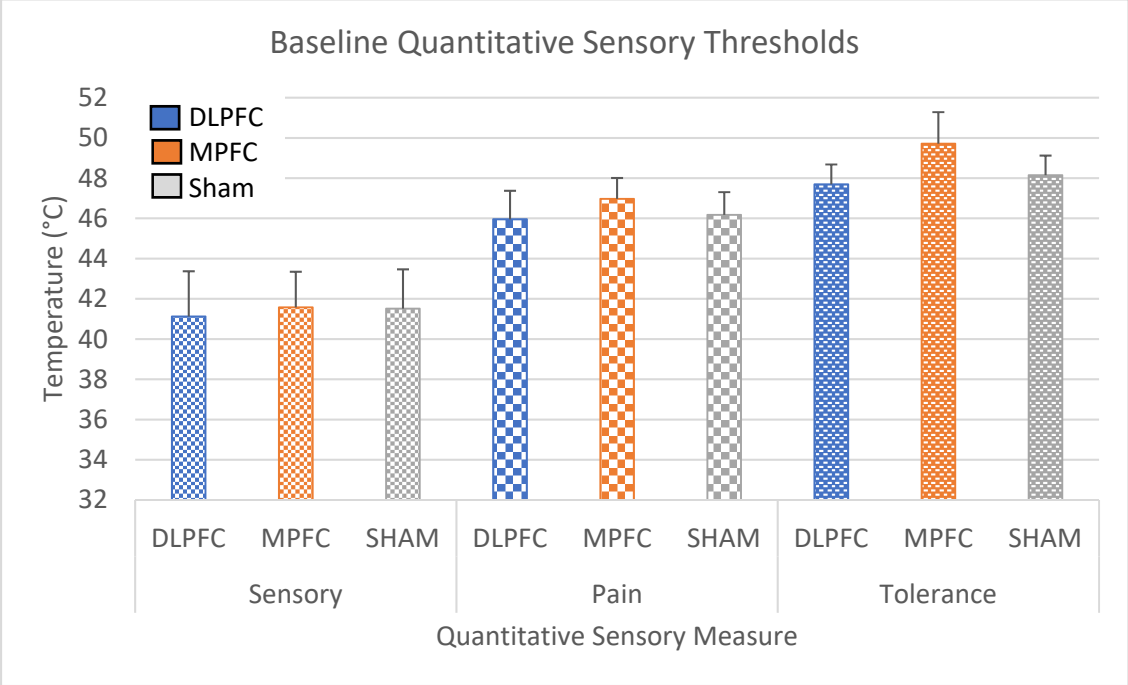

Figure 5.2 Patients Baseline Quantitative Sensory Testing Thresholds. **Pain and Tolerance Quantitative Sensory Thresholds prior to any intervention. Error bars show standard deviation. There was no difference between individuals assigned to different groups on quantitative sensory testing thresholds.**

Pre-TMS General Linear Model Results

Early Phase: Overall subjects showed positive activation in during the early phase of pain in the bilateral insula, right thalamus, anterior cingulate, right dorsolateral prefrontal cortex, right caudate, right putamen, right postcentral gyrus, anterior cingulate, SMA, cerebellum (vermis, areas VI, VIII) and areas of the brainstem corresponding to the PAG and RVM (Figure 5.3A). Decreases in activity were found in the bilateral SMA, left superior occipital gyrus, left pre and post central gyrus and left secondary somatosensory cortex. ($p<0.005$ two-sided, all clusters $p_{FWE}0.05$). Clusters sizes and coordinates in Table 5.2.

Late Phase: During the late phase of pain, we found significant positive activation only in the right cerebellum, extending to the lingual gyrus. (Figure 5.3B) Decreases in activity were found in the left primary visual cortex and superior and middle occipital gyrus. ($p<0.001$ two-sided, all clusters $p_{FWE}<0.05$).

There were no significant differences between the groups prior to TMS in either the early or late phase of the brain response to pain.

Rating Block: Patients had robust activation in response to the rating task. In order to aid in interpretability of the findings, the p value threshold was raised to a voxelwise level of $p<0.001$ (corresponding to a $q_{FDR}<0.01$), and only clusters made up of more than 65 voxels ($p_{FWE}<0.05$) were considered (Figure 5.3C). Patients had significant clusters of positive activation in the visual cortex, left motor cortex, left sensory cortex, left SMA, right cerebellum. Patients had significant negative activation in the left and right: cuneus, precuneus, superior temporal gyrus, right

secondary somatosensory cortex, left middle cingulate cortex, left middle occipital gyrus, posterior cingulate cortex, lingual gyrus, right: precentral gyrus.

There were no significant differences between the groups prior to TMS in the brain response to the rating task.

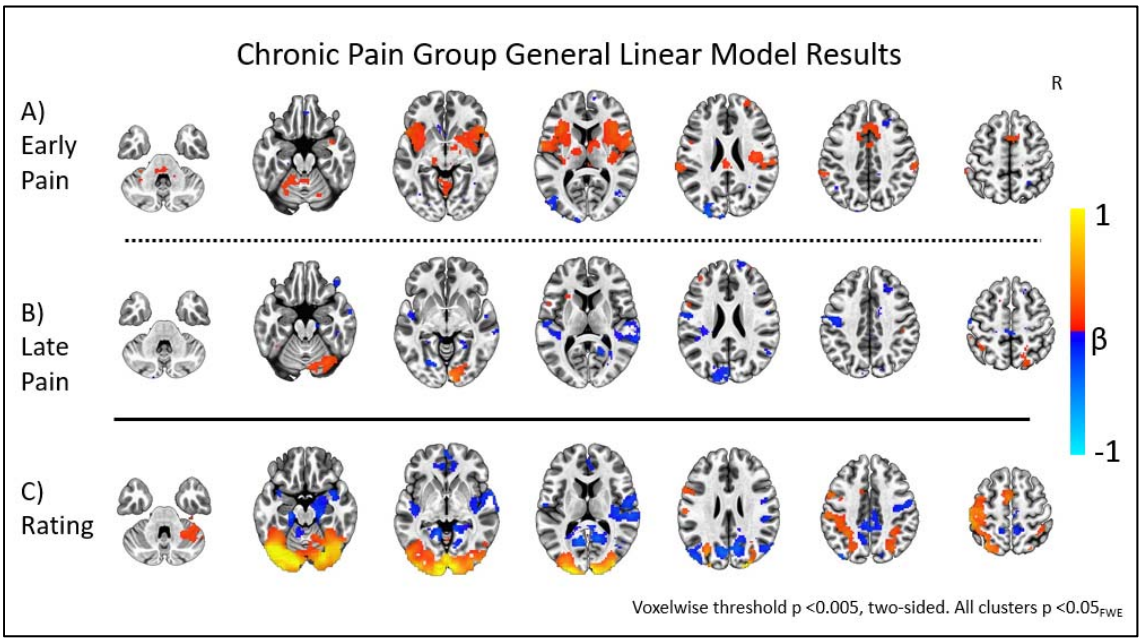

Figure 5.3 General Linear Model Results for Pain and Rating in Chronic Pain Group. A) Shows the early phase response to pain, which is thought to primarily reflect the sensory processing associated with the pain stimulus. Activation is found in the bilateral insula, thalamus, somatosensory regions, brainstem and cerebellum. B) Shows activation related to the late phase of pain processing. Significant activation was found only in the right insula. Many areas, such as the bilateral secondary somatosensory cortex showed decreases activation, reflecting a lower BOLD response during painful, compared to mild, thermal stimulus. C) Shows areas associated with the rating task, which is performed with the right hand. As expected, activity was found in the visual cortex, as well the left motor cortex, and right cerebellum.

Table 5.2 Patient's Coordinates of Peak Activation During Pain and Rating

	Size	Peak T Score		
		x	y	z
Pre TMS				
Early Phase				
	2817	-60	-10.8	0.5
	1505	62.5	-0.8	5.5
	742	0	66.8	0.5
	547	-2.5	-8.2	48
	183	22.5	96.8	28
	182	35	41.8	-54.5
Late Phase				
	803	-7.5	99.2	0.5
	506	0	29.2	58
	418	17.5	96.8	30.5
	320	-70	26.8	-2
	317	55	6.8	28
	174	42.5	31.8	10.5
Rating Block				
	10419	30	79.2	-17
	4996	12.5	61.8	10.5
	379	2.5	-0.8	58
	360	0	-55.8	-4.5
	336	62.5	14.2	8
	255	47.5	84.2	28
	248	-55	69.2	30.5
	204	12.5	46.8	-2

Relationship between Pain and Opiate Dose on the Brain Response to Acute Pain

We were able to replicate and extend our previous findings regarding the relationship between the Brief Pain Inventory and the brain response to acute thermal pain. Specifically, in this sample of patients we found that higher levels of current pain were associated with similar decreased responses during the early phase of pain in the right insula, as well as the left insula and bilateral hippocampus (Figure 5.4B, compare with Figure 4.4B). These effects are present during the early phase of pain processing which is most consistent with the analyses of pain performed in Chapter 4.

For prescription opioid dose, we again found that higher rates of prescription opioid use were associated with increased brain responses to pain (Figure 5.4A), however the pattern of increases differed somewhat from that seen in Chapter 4. We found in this sample that higher MME values were associated with increased activity in response to the early phase of pain in the left and right middle temporal gyrus, right parahippocampal gyrus and right hippocampus.

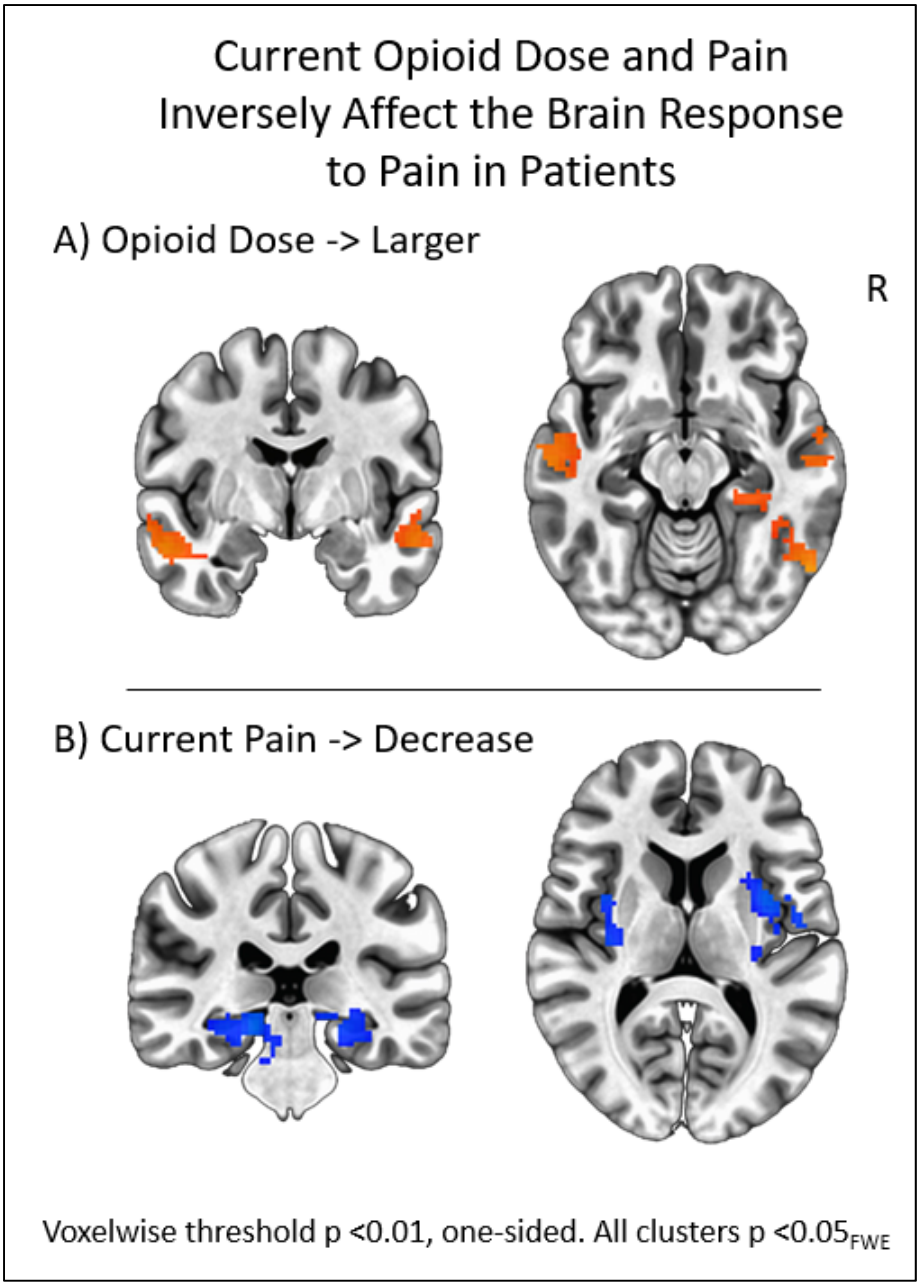

Figure 5.4 Relationship Between Pain Response and Clinical Variables. A) Shows areas in which there was a positive relationship between the prescribed dosage of opioids (morphine milligram equivalents) and the brain response to the early phase of pain. These areas included the left and right middle temporal gyrus, as well a the right parahippocampal gyrus and hippocampus. B) shows areas in which there was a negative relationship between current pain, evaluated at the beginning of experimental procedures and the brain response to pain. Significant areas include the bilateral insula and amygdala.

Behavior Correlations. There is also an opportunity to examine correlations between patient related variables and measures used within the study itself. For example, there is a negative correlation between MME values and the Baseline Measure of Pain on the QST (Figure 5.5, p = 0.015, r=0.61).

Notably there was no relationship between the painful temperature used in the scanner and the MME (r = 0.04), nor was there a relationship between the QST measure of pain and the high temperature used in the scanner (r = -0.04). This suggest that the capsaicin model used here may be probing additional measures of pain processing compared to the unsensitized pain thresholds measured from quantitative sensory testing.

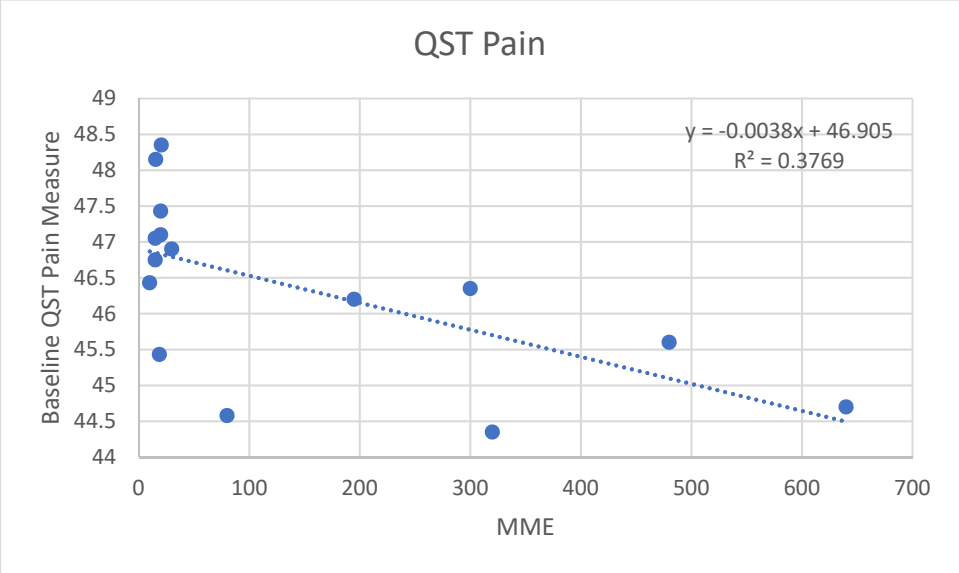

Figure 5.5 Relationship between Opioid Dosage and Quantitative Testing Pain Threshold. As individuals were prescribed more opioids, here measured in morphine milligram equivalents, this was associated with significantly (p <0.05) lower pain thresholds as measured by Quantitative Sensory Testing (QST).

Pre vs Post TMS

The sample of subjects is currently too small to support a rigorous examination of changes before and after the intervention. Nevertheless, it is still possible to examine the data for consistency with the results of Chapter 3 in healthy controls.

Behavioral Data. Table 5.3 shows the relative effect sizes of these single interventions, including data from healthy controls. Also shown are the effect sizes from the original study on theta burst stimulation in the motor cortex. For self-report data MPFC stimulation using cTBS appears to have consistent positive (pain-relieving) effects in both healthy controls and patients. Notably, in this patient population it appears that DLPFC stimulation with iTBS has similar effect sizes to MPFC stimulation, which may have important implicates for continued treatment development.

For QST measures, the results are somewhat more mixed. In healthy controls MPFC stimulation was effective in elevating pain and tolerance thresholds, whereas these effects are nearly diminished in this patient population.

Table 5.3 Effect Sizes Pre vs Post TMS on Pain Measures and MEP size. The top portion of the table shows the effect sizes (*Cohen's d*) associated with pain measures from Chapter 3 and 5. In healthy controls, cTBS delivered to the MPFC is consistently associated with the largest effects. In Patients, the results are mixed. In self-report measures, both types of active stimulation have larger effect sizes compared to sham stimulation. For Quantitative Sensory Testing, only MPFC stimulation was associated with positive effect on pain thresholds. Lower Half: The magnitude of effects is similar to conventional paradigms (10 and 1Hz) but smaller than the effects found in early studies using theta burst on the motor cortex. Negative numbers indicated increased self-reported pain or lower pain thresholds. †Data from Maeda, et al 2000 *Data from Huang et al, 2005.

Effect Size of TMS on Pain Measures

Healthy Controls	Self-Report		Quantitative Sensory Testing	
	Intensity	Unpleasantness	Pain	Tolerance
iTBS	0.22	0.28	0.27	-0.09
cTBS	0.92	0.85	0.74	0.55
Sham	0.47	0.28	-0.01	0
Patients				
iTBS	0.69	0.95	-0.21	-0.17
cTBS	0.88	0.49	0.19	-0.57
Sham	0.27	0.33	-0.61	-0.58

Effect Size of Motor Cortex TMS

	Peak Change in MEP
10Hz	0.71†
iTBS	1.85*
1Hz	0.9†
cTBS	1.76*

fMRI data. Currently the sample size is too small to support the examination of changes following a specific TMS stimulation method within the fMRI data. As was done in Chapter 3, these data were examined using a model that incorporates timepoint (pre vs post), group (DLPFC, MPFC, sham) and pain response phase (early, late). There were no significant changes pre compared to post TMS, nor were their main effects or interactions.

Discussion

Despite higher levels of pain relative to the healthy control sample in Chapter 3, all individuals suffering from chronic pain were able to complete the study procedures. These data provide important feasibility information for studies seeking to combine behavioral measures, neuroimaging and brain stimulation into a single comprehensive protocol.

One question that often emerges in studies that combine TMS with chronic pain conditions is related to tolerability. Repetitive TMS is somewhat uncomfortable, however, no individual in this study withdrew due to TMS procedures. This is an important step in showing the feasibility of using TMS in this type of clinical population. Though performed in a different clinical population, cTBS has been successfully delivered to the MPFC in nearly 200 subjects over 612 as of June 2018. These findings support this novel target as a feasible location to perform stimulus (Hanlon, Philip et al. 2019).

Beyond the feasibility of performing these TMS procedures, we also highlight the effectiveness of the fMRI protocol. Despite delivering less than half of the number of pain events (135 total, compared to 304 in Chapter 4) we were able to show significant brain responses in areas of the pain matrix. Furthermore, these effects were significant through key pain regions of the brainstem such as the PAG and RVM, which are often unexamined due to

field of view limitations or high levels of physiological noise. By combining multiband (Feinberg, Moeller et al. 2010) imaging with multiecho data collection and processing (Kundu, Inati et al. 2012, Kundu, Voon et al. 2017) we can examine these areas that are involved with the endogenous opioid response. As we continue to collect these data, we will be able to answer new questions about how these small, but critically important regions area associated with pain levels or opioid usage.

Though the early phase to pain responses show spatial similarity those seen in healthy controls in Chapter 3, reflecting primarily the sensory inputs, the late phase responses appear to differ. Specifically, there is a lack of late phase activation to pain, and instead substantially more decreased activation, such that the mild stimulus was associated with greater activity than the painful stimulus. Though this sample is only a third of the size relative to the healthy controls, these results are significant at a threshold of $p < 0.01$. This suggest that the more evaluative processes associated with pain is dysregulated in these individuals, while sensory processing remains intact. Further subjects are required in order to evaluate this hypothesis. The rating period showed the expected pattern of activation, which suggests that the effects are not due to some post pain physiological change that affects the BOLD signal.

For the early phase of pain processing we were able to replicate clinically relevant correlations between current pain and the brain response to experimental pain found in Chapter 4. Despite being an entirely independent sample, with more heterogeneity in the source of their chronic pain, the level of their pain assessed at the beginning of the experimental visit was associated with decreased responses to the pain task in the left insula. In this sample these results extended to include the right insula, as well as the bilateral amygdala. In the thermal pain task used here the amygdala was not directly activated by the stimulus, however it

is known to play a role in pain processing (Simons, Moulton et al. 2014), and its role in affective processing may a particularly important role in chronic pain (Veinante, Yalcin et al. 2013).

While it is still too early to examine the significance of these TMS interventions in this population, the early data is encouraging. The self-report effects sizes are consistent with findings from healthy controls in Chapter 3, however the QST data are less so. Specifically, only the effect size for QST pain thresholds after MPFC stimulation is in an analgesic direction. It is possible that these two separate measures of pain processing, one performed with an adapted form of allodynia, and the other performed on the non-sensitized wrist are capturing separate aspects of pain processing. There is some evidence to support this, as MME measures were correlated with QST pain thresholds, but no such correlation was found with the '7/10' thresholds used when scanning.

With the current sample, both forms of active stimulation appear to be outperforming sham, which further supports performing this work in a chronic pain population, rather than exclusively developing the treatment in healthy controls. Further work is required to determine which of these interventions will be most effective.

The Next Steps in Developing a Brain-Based Treatment for Pain

These findings highlight the validity of exploring rTMS as a tool in the fight against pain and the opioid overdose crisis. A number of steps remain before large scale clinical trials can begin. These can broadly be divided into two categories – those evaluating the pain processing and the methods developed as analgesic treatments.

A better understanding on pain processing is essential in the development of a treatment that is targeted to specific areas. As it is clear that the pain matrix is sensitive but not specific to

pain (Ochsner, Zaki et al. 2008, Iannetti and Mouraux 2010, Mouraux, Diukova et al. 2011), further work is required to build better brain region based hypotheses. In addition many studies of pain processing have failed to evaluate the entire brain, a problem they share in common with the brain imaging field at large (Vaden, Gebregziabher et al. 2012). With recent advances in neuroimaging methods these concerns regarding sample rate and voxel size are mitigated, which will enable the field to regularly obtain actual whole-brain data, particularly capturing the important brainstem and cerebellum regions.

These studies of pain processing must also consider chronic pain and the effects it has on the pain experience. While there is growing evidence that chronic pain alters both functional and structural aspects of the brain (Kregel, Meeus et al. 2015), there remain questions about the effects of pain etiology, treatments and the direction of causality. In order to answer these questions, larger studies, comparing multiple pain conditions are needed. This information will enable better models for the targeting of non-invasive brain stimulation methods.

For brain stimulation, the next steps will require studies that move beyond this proof-of-principle work and explore the treatment of pain in a manner that matches current depression treatments. In clinical settings, a response to treatment only occurs after multiple weeks of stimulation (George, Taylor et al. 2013, Schulze, Feffer et al. 2018). Future studies should consider examining the cumulative effects of multiple stimulation sessions to reduce the risk of false negative findings.

For depression there is emerging interest in accelerated treatment strategies, that deliver multiple session per day, obtaining rapid effects (Williams, Sudheimer et al. 2018). The severity of the opioid crisis, the risks of tolerance, opioid induced hyperalgesia and overdose in addition to the difficulties associated with living with chronic pain make it amenable to such treatment

methods. It may be appropriate to build research programs that move towards these accelerated protocols in order to deliver rapid pain-relieving effects, which may reduce participant burden and improve retention.

The Future of Brain Stimulation Technology

While this work primarily focused on the use of methods that employ electromagnetism to probe and modulate the brain, the field has not limited itself to these techniques. Several new methods have been developed which may be able target the regions involved in pain processing with hitherto unavailable accuracy or modulatory effects.

In keeping with the use of electrical principles, the first we will discuss is non-invasive stimulation using temporally interfering electrical fields (Grossman, Bono et al. 2017). This technique is similar to prior work which sought to use the summation of magnetic fields (Mocanu, Weiss et al. 2004), but instead uses electrical stimulation. Specifically, high frequency stimulation (~2kHz), which has little to no effect on neural processing, is delivered via multiple electrodes positioned at various locations on the scalp. Key to the temporal interference principle is that the stimulation frequencies are offset from one electrode to the other by some small amount, such as 1Hz. Thus, the electrical fields interfere at some location deeper within the brain, producing an effective stimulation frequency of 1Hz. Though a number of steps remain before translating to use in humans, it is possible that this technique could be used to target inhibitory stimulation to one of the medial or lateral thalamic locations which relay the nociceptive signal to the cortex.

Another promising technological development for brain stimulation moves entirely beyond electromagnetic principles and instead employs sound. Specifically, ultrasound which is made up

of frequencies greater than 20,000 kilohertz, beyond the range of human hearing. Research with ultrasonic methods has primarily followed two paths. The first considers ultrasound as a method to alter cortical excitability directly (Legon, Sato et al. 2014). This ultrasonic stimulation can be at the cortex, where there is evidence that it can modulate ongoing brain activity (Legon, Sato et al. 2014, Mueller, Legon et al. 2014) in the somatosensory cortex. This shallow stimulation makes it similar to existing transcranial magnetic stimulation. One benefit of ultrasound compared to TMS, is that is nearly imperceptible and not associated with discomfort or muscle twitches. While this makes the creation of sham stimulation easier, it has the more important benefit of being more comfortable and tolerable for patients. In addition, ultrasound can be focused to deeper areas of the brain, such as the thalamus (Legon, Ai et al. 2018). Direct stimulation and modulation with ultrasound may be a powerful new technique to attenuate the pain experience that is able to build upon the decades of prior work with magnetic fields.

The other view of ultrasound sees it as a precise tool which can be used to release or activate pharmacological agents within circumscribed areas of the brain (Airan, Meyer et al. 2017, Wang, Aryal et al. 2018). This method, known as 'uncaging' or 'ultrasound gating' uses nanoparticles to encapsulate a pharmacological agent. When stimulated with ultrasound, the drug is released. By taking advantage of the focality and depth of ultrasound, a drug can be released in a specific deep brain location while remaining inert throughout the rest of the brain and body. These methods have demonstrated success, causing the cessation of seizures in rodents (Airan, Meyer et al. 2017) and modulating distributed brain activity via focal drug delivery (Wang, Aryal et al. 2018). Continued development of this tool would provide an opportunity to reevaluate the entire pharmacopeia, as concerns regarding dosage and off-target effects would need to be reconsidered. While considerable work is required prior to usage in

humans, this technique also holds promise for pain relief. It is possible that this could be another powerful method for analgesia, by pharmacologically modulating specific thalamic or brain stem regions involved with pain processing.

It is clear that there is great promise for brain stimulation as a treatment for pain. While several methods and targets have been validated, there remain extensive opportunism for more research. It is essential to continue to explore cortical and subcortical targets in both healthy controls as well as individuals with chronic pain. An ideal brain-based treatment for pain will serve both populations. For healthy individuals receiving their first serious injury, it must provide analgesia and reduce the current clinical dependence on prescription opioids. For those who have been suffering from chronic pain for years, this treatment needs to assist with pain relief, such that these individuals can reduce their prescription opioid intake and regain their life.

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